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CYTOLOGIC CHANGES IN BRONCHOGENIC CARCINOMA FOLLOWING TREATMENT WITH NITROGEN MUSTARD (METHYL-BIS [β-CHLOROETHYL] AMINE)

EDWARD A. GAENSLER, M.D.

DONALD G. McKAY, M.D.

PAUL F. WARE, M.D.

AND

JOSEPH P. LYNCH, M.D.

BOSTON

THE first study of the effects of mustard gas on tumor tissue was made by Berenblum in 1929.[1] He attempted to accelerate the formation of tar-induced tumors in mice by increasing the local blood supply to the skin with mild irritants. Dichlorodiethylsulfide in 1 : 1,000 dilution was one of the irritants used. This chemical added to the carcinogenic coal tar, however, almost completely inhibited tumor formation. This protection was exerted even if the mustard was not added until the twelfth week of treatment. Slightly larger concentrations of the mustard gas solution could cause destruction of tumor tissue.[2] This characteristic appeared to be peculiar to mustard gas, since other skin irritants, with the exception of cantharidin, did not exert a protecting influence.[3] Systemic action was demonstrated by the fact that glycolysis and respiration of slices of sarcoma in vitro were depressed in the presence of anticarcinogenic substances, such as mustard gas, dichlorodiethylsulfone and cantharidin.[4]

The nitrogen mustards were synthesized as potential offensive warfare agents and proved to have action similar to that of mustard gas, but, unlike the latter, they were water soluble and therefore more easily handled. Peters and collaborators[5] at Oxford University, Dixon and Needham[6] at Cambridge University and Gilman and Philips[7] and

From the Thoracic Surgery Service and the Mallory Institute of Pathology of the Boston City Hospital.

1. Berenblum, I.: J. Path. & Bact. **32**:425, 1929.
2. Berenblum, I.: J. Path. & Bact. **34**:731, 1931.
3. Berenblum, I.: J. Path. & Bact. **40**:549, 1935.
4. Berenblum, I.; Kendal, L. P., and Orr, J. W.: Biochem. J. **30**:709, 1936.
5. Peters, R. A.: Nature, London **159**:149, 1947.
6. Dixon, M., and Needham, D. M.: Nature, London **158**:432, 1946.
7. Gilman, A., and Philips, F. S.: Science **103**:409, 1946.

authors in this country quoted by them have recently published reviews of the work done on these substances during the war years. The primary objective of these extensive and well organized studies, namely, the discovery of an effective antidote or "competitive fixative" in the treatment of mustard gas poisoning, was not as successful as, for example, the discovery of BAL (2,3-dimercaptopropanol) for the arsenical group of war gases. It was established early that after exposure to these mustard compounds a portion of the substance is almost instantly "fixed" to the skin,[8] and the remainder is absorbed where it, too, is "fixed" within a few minutes.[8] The great affinity of these chemicals for a wide variety of enzymes and tissue nuclear proteins [9] makes it appear that introduction of "competitors" in the treatment of poisoning is doomed to failure unless treatment is instituted almost coincidentally with exposure.[10] On the other hand, much knowledge was accumulated during this time concerning the selective action of these drugs on rapidly proliferating tissues and their nucleotoxic action, as well as their in vitro and in vivo inactivation of enzymes.

The affinity shown by the mustards for proliferating tissues, such as the hemopoietic and lymphatic systems and the intestinal epithelium, has never been shown by any other drug. This affinity, as well as Berenblum's work on its anticarcinogenic properties, suggested further study in this direction. Friedenwald and associates,[11] while investigating the effects of nitrogen mustards on the mammalian cornea, demonstrated almost complete inhibition of mitotic activity even when the substance was used in dilutions only one one hundredth of those which cause clinical signs of intoxication. Nucleotoxic action was demonstrated on the resting cells of the basal layers of the corneal epithelium by the occurrence of nuclear fragmentation. This effect on resting cells of rapidly proliferating tissues was further manifested by the fact that, although 50 per cent of bone marrow cells are usually in the resting stage, over 90 per cent of these cells disappeared within a short time after mustard poisoning. Such inhibition of mitosis and nucleotoxic action was demonstrated not only in the cornea and bone marrow but also in tissue cultures of a large variety of normal and neoplastic cells.[12] In growing embryos these processes appeared to take

8. Black, S., and Thomson, J. F.: Proc. Soc. Exper. Biol. & Med. **63**:460, 1946.

9. Herriott, R. M.; Anson, M. L., and Northrop, J. H.: J. Gen. Physiol. **30**: 185, 1946.

10. Collumbine, H.: Nature, London **159**:151, 1947.

11. Friedenwald, J. S.; Buschke, W., and Scholz, R. O., in Moulton, F. R.: Approaches to Tumor Chemotherapy, Monograph, American Association for the Advancement of Science, Lancaster, Pa., Science Printing Company, 1947.

12. Karnofsky, D. A.; Burchanal, J. H.; Ormsbee, R. A., and Cornman, I.: Cancer Research **7**:50, 1947.

place selectively in proliferating areas only.[13] Nuclear fragmentation as well as karyolysis are described in great detail by Friedenwald and associates.[11] Here, as following roentgen irradiation, changes in osmotic pressure and permeability of cell membranes appear to play a major role.

Mustard gas derivatives were able to induce mutations in Drosophila [14] and Neurospora,[15] a property heretofore thought to be peculiar to ionizing radiation. In fact, so strikingly parallel were the observed actions of the mustards and radiation that it was possible to compute that 100 moles of one of the nitrogen mustards exhibited the activity of one roentgen ray ion or 50,000 photons of ultraviolet rays.[11]

Selective cytotoxic and nucleotoxic action on neoplastic tissue was therefore expected. Karnofsky and co-workers [12] observed that a "take" of mouse leukemia was prevented if donor mice had been previously treated with nitrogen mustards. Of several mustard derivatives tested, the methyl-bis and the tris compounds proved to have a definite "cytocidal dose" for these leukemias. Cornman and Ormsbee [16] noted that in rollertube cultures the observable response to methyl-bis (β-chloroethyl) amine (hereafter referred to as HN2) was quantitatively the same for normal and cancerous tissue but that the response occurred at different dose levels. Recently Karnofsky and co-workers [12] were able to kill selectively sarcoma 180 growing on the chorioallantoic membrane of the chick embryo without affecting the embryo's growth.

Gilman and Philips [7] were the first to treat human subjects for neoplasms. Some time later Jacobson and co-workers [17] independently began to treat similarly the neoplastic disorders of the hemopoietic and lymphatic systems. Since then there have been numerous reports on the efficacy of the nitrogen mustards in the treatment of Hodgkin's disease, leukemia and allied disorders. Promising remissions of Hodgkin's disease have been reported by many clinicians. Good results have also been obtained in the treatment of slowly growing lymphosarcoma, chronic leukemia and erythremia. Unfavorable results are reported in acute leukemia, rapidly growing lymphosarcoma and melanocarcinoma.[18] It is the consensus at the present time that nitrogen mustard

13. Bodenstein, D.: Cancer Research **7**:49, 1947.

14. Horowitz, N. H.; Houlahan, M. B.; Hungate, M. G., and Wright, B.: Science **104**:233, 1946.

15. Auerbach, C., and Robson, J. M.: Nature, London **158**:878, 1946.

16. Cornman, I., and Ormsbee, R.: Federation Proc. **6**:390, 1947.

17. Jacobson, L. O.; Spurr, C. L.; Smith, T. R., and Dick, G. F.: M. Clin. North America **31**:3, 1947.

18. (*a*) Alpert, L. K., and Peterson, S. S.: Bull. U. S. Army M. Dept. **7**:187, 1947. (*b*) Sherry, M.: South. M. J. **41**:118, 1948. (*c*) Rhoads, C. P.: J. A. M. A. **131**:656, 1946. (*d*) Taffel, M.: Yale J. Biol. & Med. **19**:971, 1947. (*e*) ApThomas, M. I. R., and Collumbine, H.: Lancet **1**:899, 1947. (*f*) Jacobson and others.[17]

therapy is indicated when the neoplasms just mentioned become refractory to roentgen radiation or the disease is too widely disseminated for effective irradiation of the involved regions. Promising results have also been reported in the treatment of Boeck's sarcoid,[19] mycosis fungoides,[20] neuroblastoma, Ewing's tumor and rhabdomyosarcoma.[21]

Bronchogenic carcinoma has been treated with HN2 with favorable results,[18c] although so far only 2 cases in which an anaplastic type was treated thus have been reported in the literature.[22] In both patients the carcinoma was far advanced, both received roentgen ray treatment concomitantly, and in both results appeared to be promising.

Only a few isolated studies of cytologic changes of neoplastic tissue following mustard treatment in man have been reported. Osborne and associates [20b] obtained biopsy specimens of previously involved areas of skin from 2 patients with mycosis fungoides eleven and thirty days after treatment, respectively. In both instances there was no involvement of the tissues and no studies of cytologic changes were possible. We have had similar experiences in a number of cases discussed in the following section of this paper. Alpert and Peterson [18a] studied lymph nodes excised from 2 patients with Hodgkin's disease three days after completion of the HN2 course. He found necrosis of reticulum cells, eosinophilic granulocytes and Reed-Sternberg cells. Sherry [18b] made routine pretreatment and post-treatment biopsies of marrow in cases of Hodgkin's disease, and found pyknosis of nuclei of lymphocytes as well as clumping of cells. No cytologic studies were reported on either of the 2 cases of HN2-treated bronchogenic tumors referred to in the foregoing paragraph.

MATERIALS AND METHODS [23]

During the course of a study of a large series of patients with inoperable or metastatic bronchogenic carcinoma treated with HN2 it was noted that certain patients responded more readily to this type of therapy than did others. Furthermore, though the degree of response was frequently related to the degree of anaplasia as judged from the microscopic appearance of the tumor, this was by

19. Snider, G. E.: South. M. J. **41**:11, 1948.

20. (*a*) Philpott, O. S.; Woodburne, A. R., and Waldriff, G. A.: J. A. M. A. **135**:631, 1947. (*b*) Osborne, E. D.; Jordon, J. W.; Hoak, F. C., and Pschierer, F. J.: J. A. M. A. **135**:1123, 1947. (*c*) Taffel.[18d]

21. Cowdry, E. V.: J.A.M.A. **135**:1067, 1947.

22. Karnofsky, D. A.; Craver, L. F.; Rhoads, C. P., and Abels, J. C., in Moulton, F. R.: Approaches to Tumor Chemotherapy, American Association for the Advancement of Science, Lancaster, Pa., Science Press Printing Company, 1947. Alpert and Peterson.[18a]

23. The nitrogen mustard used in this study was supplied by Merck & Co., Inc., through the agency of Dr. D. A. Karnofsky, of the Committee on Growth, of the National Research Council.

no means always the case.[24] It was felt that cytologic studies of tumor tissue before and after treatment might aid in explaining these differences in response and aid in an understanding of the mode of action of this drug. This palliative treatment was instituted only in cases in which there was microscopic evidence of bronchogenic carcinoma but the patients were unsuitable for surgical treatment. The entire series with its clinical aspects is being presented elsewhere.[24]

The primary difficulty with such an undertaking was the inability to get biopsy specimens at the proper times after treatment. In some cases the original biopsy specimen was obtained at exploratory thoracotomy and there was no possibility of obtaining further biopsy specimens after HN2 treatment. In others the patients were seen some time after pneumonectomy with hepatic, cerebral or osseous metastases not accessible for biopsy. In still others the patients' general condition precluded repeated bronchoscopic examinations and removal of specimens.

In 12 cases tissue was available during or after treatment. In 4 of these cases although pretreatment and post-treatment specimens were obtained, the latter showed no tumor tissue histologically in an area previously known to contain tumor. Two patients had extensive involvement of the trachea at the time of the first bronchoscopy. A biopsy specimen was obtained with ease from each. Six days after treatment in one patient and eight days after treatment in the other no tumor could be observed on bronchoscopy and specimens taken from the previously involved areas, which now grossly appeared fibrotic, showed no tumor cells on microscopic observation and had the appearance of normal bronchial mucosa. There was no evidence of fibrosis or necrosis. Two other patients were seen about one year after pneumonectomy, at which time they showed great enlargement of cervical and submental lymph nodes. Biopsy specimens, obtained without difficulty, showed metastatic carcinoma in both. Five days after onset of treatment in one patient and twelve days after onset of treatment in the other the enlargement of the lymph nodes had grossly disappeared. In 1 case a specimen obtained after prolonged exploration of the submandibular region revealed fat and striated muscle only and there was no evidence of tumor, fibrosis or necrosis. In the other case the surgeon was unable to find any tissue suggesting tumor, and biopsy of several fragments similarly showed no remnants or other evidence of tumor. Since it is inconceivable that there should be no microscopic evidence of tumor, necrotic tissue or fibrosis so short a time after treatment, it must be presumed that the neoplastic tissue had shrunk to such an extent that the surgeon was unable to obtain a specimen of it at the correct place. These cases will not be discussed further.

In 8 cases adequate biopsy specimens were obtained after onset of treatment. In 1 case treatment was started while the patient appeared to be in terminal coma. He died ten days after the onset of treatment, and the specimen was obtained at postmortem examination. Another patient was treated immediately after exploratory thoracotomy and died ten days after termination of the HN2 course of total empyema and massive sepsis. Again tissue was obtained at postmortem examination. The third patient had a chain of large inguinal lymph nodes from which biopsy specimens were obtained seven days after treatment and again seven days later, and further tissue was obtained at postmortem examinations two months later. The fourth patient was subjected to cavernostomy for a large carcinomatous abscess of an upper lobe of a lung because of a septic course and large amounts of foul sputum. The lesion was subsequently found

24. Lynch J. P.; Ware, P. F., and Gaensler, E. A.: To be published.

to be inoperable, and the patient received four courses of HN2 over the course of a year. His draining cavity offered a unique opportunity for biopsy, and specimens were taken every other day for ten days after the onset of treatment in each course. Further material was obtained at postmortem examination shortly after the fourth course of HN2. In the remaining 4 cases tumor tissue was obtained before and after treatment from cervical lymph nodes or from the primary tumor at the time of bronchoscopic examination.

EFFECTS OF NH2 TREATMENT OBSERVED IN TUMORS

Epidermoid Carcinoma (squamous cell carcinoma, grade 1).—Formation of large multinucleated cells and cells with multilobed nuclei and fragmentation and disintegration of nuclei and cytoplasm are the principle changes noted in well differentiated squamous cell or epidermoid carcinoma after treatment with HN2. An unexpected finding in this type of tumor was a slight but definite increase in the number of mitoses.

These changes are best demonstrated in 1 of our cases in which, because of the easy accessibility of the tumor at the edge of a cavernostomy wound, multiple biopsies were made on successive days after treatment. Tissue was obtained before treatment and two, five and six days after treatment. The tumor histologically was a typical well differentiated epidermoid carcinoma with pearl formation, numerous mitotic figures and nuclei which were either round or oval and fairly regular in size and contained a single large basophilic nucleolus (fig. 1*A*). Fifty mitoses were counted in an area approximately 0.5 cm. square. Two days after treatment was begun, no morphologic difference from the pretreatment biopsy specimen could be detected with the exception of a slight increase in the number of mitotic figures. Sixty were counted in an area similar in size to that of the pretreatment biopsy specimen. The fifth day specimen revealed more definite changes. The number of mitoses had increased to 69 in a similar field. Several multinucleated cells were seen. Many nuclei had enlarged, were irregular in shape and vesicular and contained large, irregular clumps of chromatin material rather than a single round nucleolus (fig. 1*B*). There was a distinct increase in the number of cells with irregular, hyperchromatic nuclei. In addition, the connective tissue stroma had become edematous, and the nuclei of the fibrocytes, particularly in regions immediately surrounding clumps of tumor cells, had enlarged and were rounded rather than elongated and spindle shaped. Numerous polymorphonuclear leukocytes were scattered throughout the edematous stroma. The sixth day specimen showed the most striking changes. The number of mitoses per 0.5 cm. square was 105, a twofold increase. Many of these mitotic figures were atypical, including some with multiple spindles and bizarre forms, with chromatin material scattered through the entire cytoplasm of giant-sized cells. A number of cells were in various stages of necrosis and disintegration. Some of these were shrunken cells with pyknotic nuclei and bright red cytoplasm. Others were large cells with a well preserved cell membrane but without nucleus and a mass of both basophilic and acidophilic debris filling the entire cell. Numerous spaces about the size of one or two cells appeared within the tumor tissue. Some of these appeared empty; others were filled with basophilic clumps and acidophilic granules. The connective tissue changes seen in the specimen of the preceding day were again noted.

In the second case of epidermoid carcinoma the post-treatment biopsy was made one week after treatment was started. There was a slight increase in the number of mitoses, and many nultinucleated cells with vesicular nuclei containing large, irregular masses of chromatin.

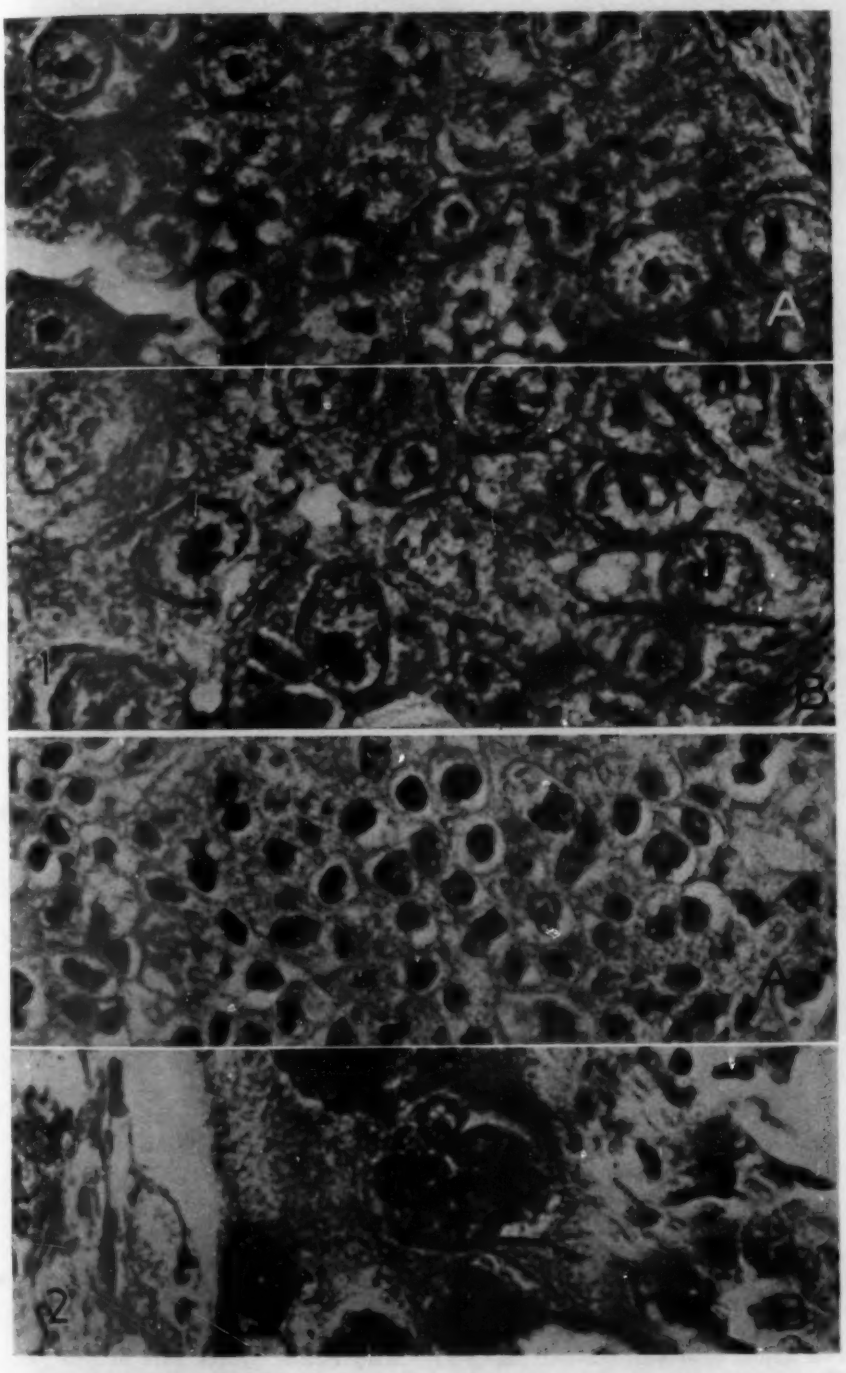

Fig. 1—Epidermoid carcinoma (case 1): *A*, before treatment. *B*, five days days after onset of treatment. × 800.

Fig. 2.—Epidermoid carcinoma (case 3): *A*, before treatment. *B*, ten days after onset of treatment. × 800.

Two biopsy specimens were obtained in the third case. Here the most striking change was the formation of enormous tumor giant cells (fig. 2 *A* and *B*). The cells had enlarged to ten to twenty times their former size. Nuclear material occupied most of the cell and consisted of as many as twenty-five to thirty darkly staining pyknotic nuclei or nuclear fragments. These cells were not found in all parts of the tumor but were prominent in most areas. There was again a moderate increase in the number of mitoses.

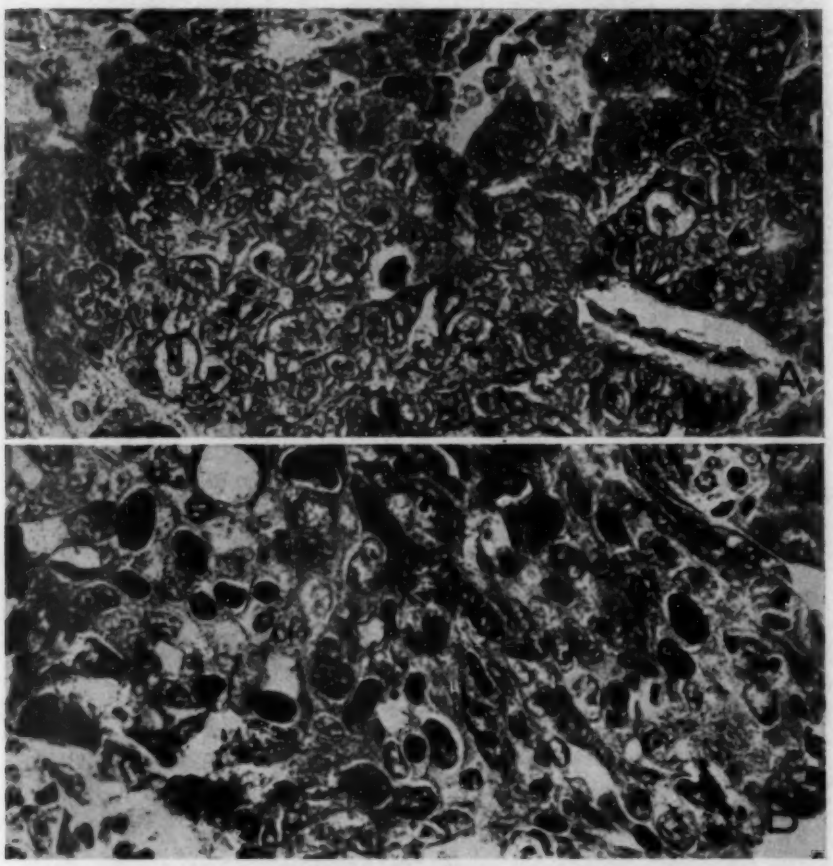

Fig. 3.—Squamous cell carcinoma: *A*, before treatment. *B*, ten days after onset of treatment. × 500.

Squamous Cell Carcinoma (squamous cell carcinoma, grade 2).—Two cases of squamous cell carcinoma were available for study. Before treatment these tumors were made up of sheets of cells with nuclei of relatively uniform size containing prominent nucleoli (fig. 3 *A*). Many mitotic figures were present. A biopsy specimen taken five days after treatment showed numerous giant-sized nuclei which were vesicular and contained large, irregular masses of chromatin. A few multinucleated cells were present, which were not seen in the pretreatment specimen (fig. 3 *B*). There was a reduction of the number of mitoses.

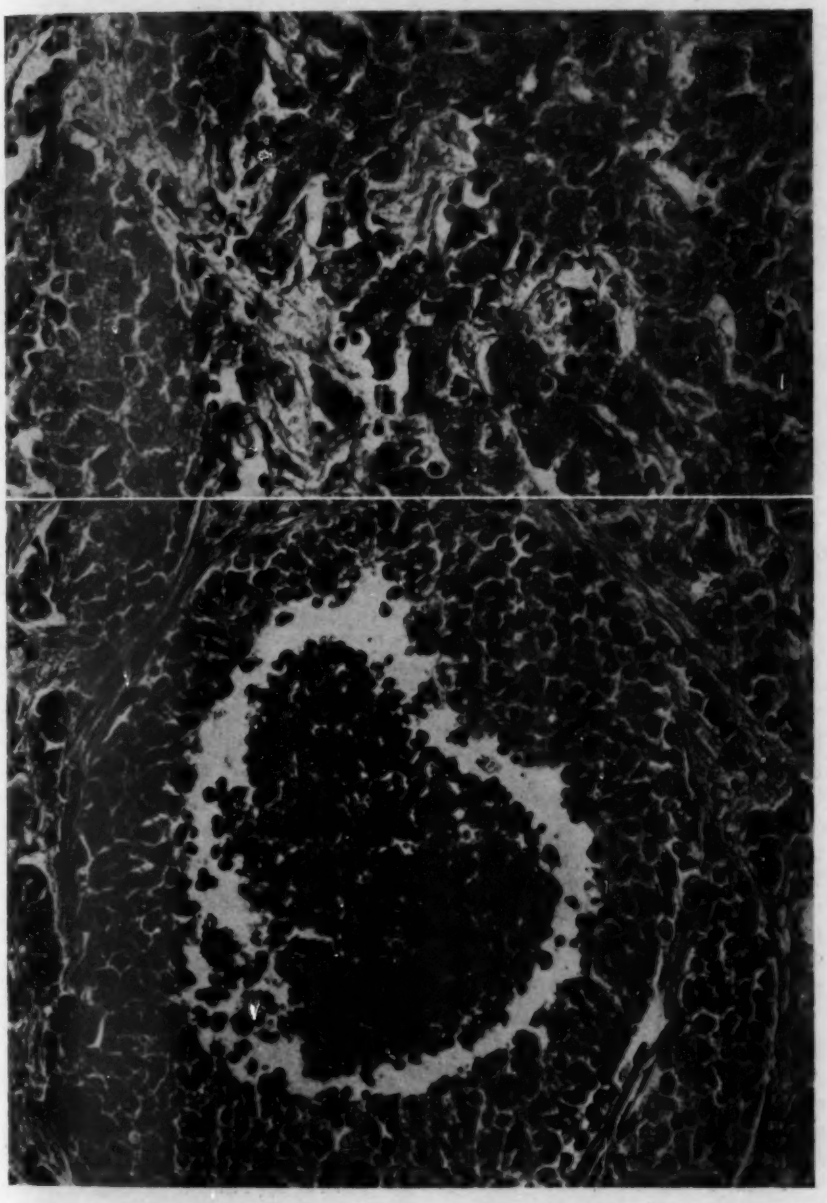

Fig. 4.—Undifferentiated carcinoma: *A*, before treatment. *B*, eight days after onset of treatment. × 400.

Undifferentiated Carcinoma (oat cell carcinoma).—Although the gross reduction in size of the tumor masses of undifferentiated carcinoma was much greater than in the well differentiated tumors, the microscopic changes were not so striking. The most prominent change was necrosis of cells with reduction of the number of mitoses. Multinucleate forms and cells with irregular nuclei were not encountered in after-treatment sections. In 3 cases of undifferentiated carcinoma, biopsy specimens were taken five and eight days after the onset of treatment. In all cases, the tumor before treatment was composed of sheets and cords of small cells with small vesicular nuclei which did not vary greatly in size and shape. They had scant amounts of cytoplasm with indistinct cell membranes (fig. 4 *A*). After treatment the tumor tissue showed a reduction of the number of mitoses. Foci of necrosis appeared in the centers of tumor areas. The nuclei became pyknotic and were fragmented. In some of these necrotic areas infiltrating polymorphonuclear leukocytes were noted (fig. 4 *B*). A less obvious change was an increase of the number of cells having an irregular, hyperchromatic nucleus.

EFFECTS OF HN2 TREATMENT OBSERVED IN TISSUES NOT INVOLVED BY TUMOR

Autopsy material was available for study in 8 cases. Five of these were in our own series. In none of these cases was death directly due to HN2 treatment. Three of the patients died within ten days after conclusion of treatment, one of total empyema and overwhelming sepsis, another of terminal cachexia, and the third was admitted in coma and did not recover. The 2 patients remaining out of the 5 died about six weeks after treatment, one of multiple metastases involving almost every organ of the body, the other of tracheal obstruction.

The 3 patients not of our own series clearly died of the toxic effects of large doses of nitrogen mustard. Connerley and Sager gave us permission to review the material from 2 of these patients. The last was treated for mycosis fungoides and also died within ten days after the conclusion of treatment.

It was thus possible to observe not only the effects of toxic doses of the drug but the effects of doses within the therapeutic range and, in 2 instances, the picture following recovery from treatment.

It cannot be stated quantitatively what constitutes a "toxic" dose and what a "therapeutic" dose. It can be said, however, that no patient who received a total of 0.4 mg. per kilogram of body weight, the standard dose recommended by the Committee on Growth of the National Research Council, showed any toxic manifestations beyond brief leukopenia. All patients who died of toxic manifestations due to nitrogen mustard received 1.5 mg. per kilogram or more.[25] On the other hand, we have given up to 3 mg. per kilogram over the course of three weeks in some cases of our own series with survival.[24]

Lymph Nodes.—Toxic doses of HN2 caused a marked alteration of the appearance of lymph nodes. The reticulum and the connective tissue were compressed, giving the nodes a fibrous appearance. This appearance may represent merely collapse of the node because of the loss of lymphocytes or a real increase of connective tissue (fig. 5 *A*). Almost all lymphocytes had disappeared, and in their place were numerous plasmacytes and cells with acidophilic cytoplasm and large nuclei (fig. 5 *B*). The latter had the general appearance of macrophages, but none were seen with phagocytosed debris within the cytoplasm.

In lower dosage HN2 did not produce effects as pronounced as those just described. Lymph follicles were present peripherally in the nodes and were

25. Connerley, H. L., and Sager, G. F.: Personal communications to the author.

made up of adult lymphocytes but were without germinal centers. The central regions of these nodes, however, had the same appearance as those receiving toxic doses.

The only nodes available from the patients who recovered from the effects of HN2 were filled with metastatic tumor. However, in one or two of these nodes numerous lymph follicles were seen and contained large germinal centers surrounded by masses of adult lymphocytes.

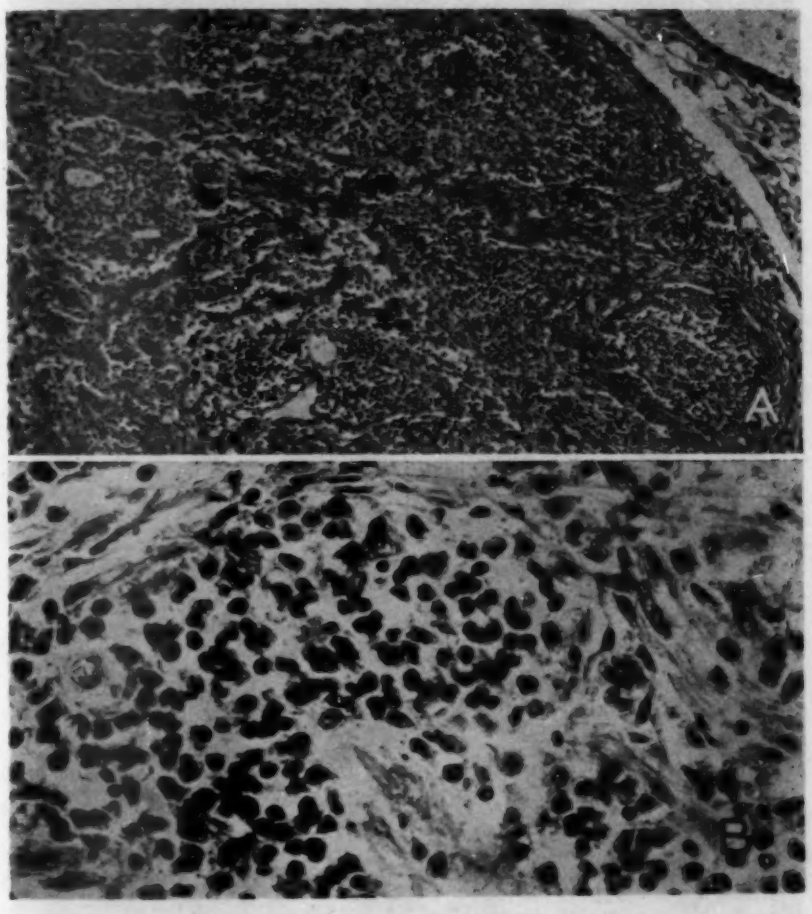

Fig. 5.—Lymph node: *A*, ten days after toxic dose of HN2; × 100. *B*, ten days after toxic dose of HN2; × 500.

Spleen.—A complete disappearance of malpighian corpuscles occurred following the administration of toxic doses of HN2 (fig. 6). A few lymphocytes could be found in the spleen. Numerous clumps of plasmacytes were seen; some of them were binucleate. In addition, there was a relatively large amount of deposited hemosiderin.

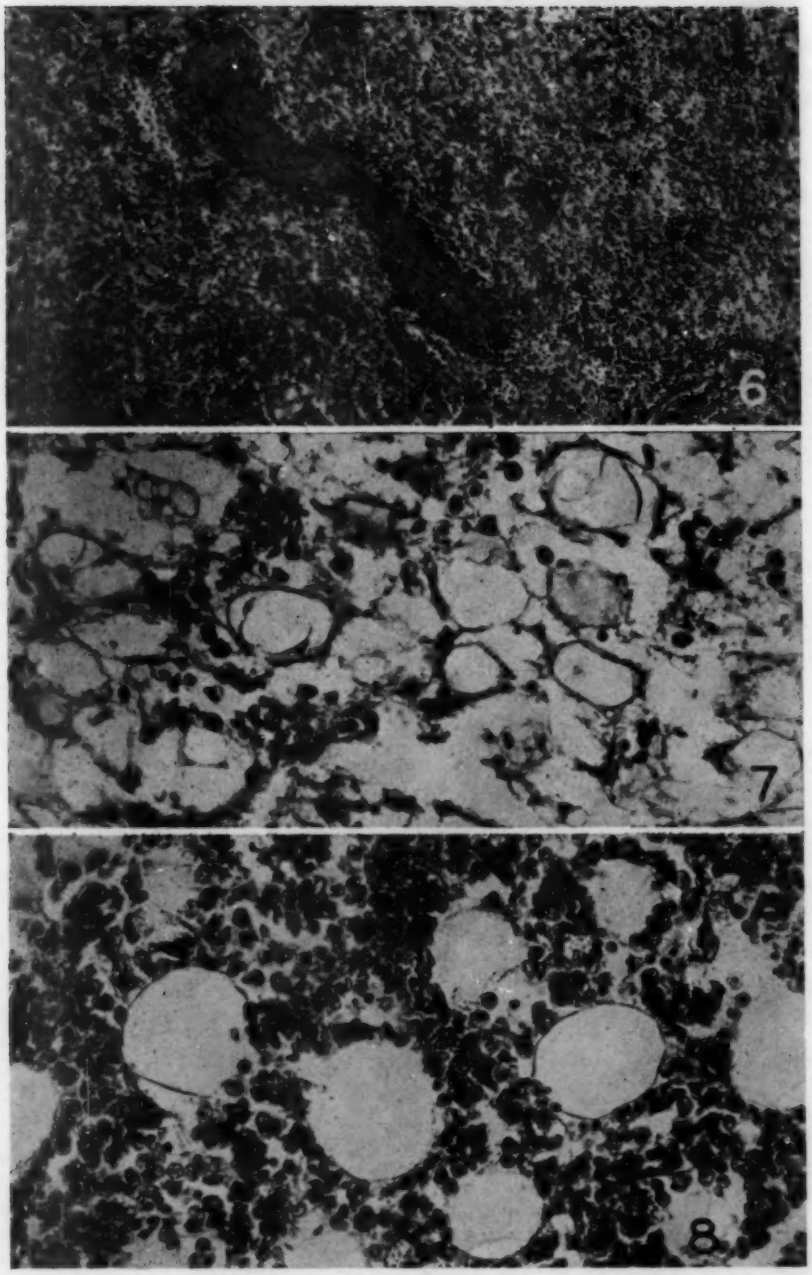

Fig. 6.—Spleen ten days after toxic dose of HN2; × 100.
Fig. 7.—Bone marrow ten days after toxic dose of HN2; × 400.
Fig. 8.—Bone marrow ten days after therapeutic dose of HN2; × 400.

Malpighian corpuscles were present in the spleens of those patients receiving lower doses of HN2, and the only abnormal finding was an increased number of plasmacytes and large deposits of hemosiderin.

Bone Marrow.—A pancytopenia occurred in patients who died after toxic doses of HN2. The only cells remaining in the marrow of these patients were small collections of plasmacytes and a few unidentifiable cells similar to those seen in the lymph nodes. The sinusoids were filled with red blood cells and acidophilic granular debris, and there were numerous heavy deposits of hemosiderin. No myelocytes, prorubricytes, rubricytes, metarubricytes or megakaryocytes could be found (fig. 7). Many histiocytes contained red blood cells within their cytoplasm.

With smaller doses the changes occurring in the marrow were not so pronounced. In 1 case the sinusoids were congested and filled with red blood cells. There were numerous clumps of prorubricytes, rubricytes and metarubricytes, and a few megakaryocytes were present. However, this marrow contained almost no mature polymorphonuclear leukocytes and only a few clusters of myelocytes could be identified. The only change, therefore, was a diminution of the white cell series and the deposition of hemosiderin (fig. 8).

The marrow of the patients dying several weeks after treatment was extremely cellular, contained little fat, and had the appearance of a normal or a slightly hyperactive marrow.

Lungs.—Two of the 3 patients who died of HN2 toxicity had multiple small pulmonary hemorrhages and 1 had rather severe pulmonary edema. No pulmonary changes were noted in the patients receiving lower doses.

Testis.—Two of the 3 patients who received toxic doses had a marked change in the tubules of the testis. The only cells remaining in these tubules were the Sertoli cells. No germinal cells were seen. One of these patients was 21 years old, but the other was over 40, and therefore the effects on the testis were difficult to evaluate. No effect on the interstitial cells was noted.

Adrenal Glands.—No morphologic changes were detected in the adrenal glands of the patients who received therapeutic doses. However, in the cases of HN2 toxicity there was a complete disappearance of lipid from the cortex.

No morphologic changes were observed in intestine, kidney, liver, thyroid gland or pancreas.

COMMENT

On reviewing the sections of tumor, two facts stand out above all others: First it must be noted that every one of the after-treatment biopsy specimens, when examined by itself, shows a picture which is frequently found in tumors which have not been subjected to toxic substances or irradiation. Nuclear fragmentation, disintegration of cells and groups of cells, focal areas of necrosis and variations of granularity and of staining reaction are all seen frequently on examination of any group of tumors of this type. Even formation of large giant cells with enormous multilobed nuclei is not unusual. Secondly it is evident that when the sections are viewed in relation to the pretreatment specimen every one of them shows a definite change in the histologic appearance of the tumor. It is interesting that the most unquestionable and striking changes occurred in the highly differentiated type of tumor. Increase of the number of mitoses, giant cell formation and nuclear and cellular

disintegration were most noteworthy in this group. On the other hand, the least pronounced changes occurred in the undifferentiated cell type where areas of necrosis are so frequently found without previous chemotherapy or irradiation. Necrosis was accepted as a change due to HN2 treatment only because there was none in the pretreatment specimen and because this offered the only explanation for the decrease in size of involved lymph nodes with treatment. The only definite change here was a decrease of mitotic activity. The gross reduction of size of tumor as well as the favorable clinical response were most marked in the undifferentiated type.[24]

It is possible that this seeming paradox can be explained by existing knowledge. Bodenstein [13] has been able to show that cells of the embryo of Amblystoma punctatum show two types of reaction to HN2 exposure, a striking increase in size of well differentiated cells and a rapid disintegration in proliferating areas. Friedenwald and co-workers [11] similarly showed formation of very large cells in the epithelial layers of the cornea when mitoses were inhibited with repeated minute doses of HN2. The differentiating areas of epidermoid bronchogenic carcinoma show giant cell formation from which further tumor growth may occur, while the more rapidly proliferating areas show necrosis. Regaud [26] felt many years ago that epidermoid or other highly differentiated tumors developed in two directions, a further growth of neoplastic tissue cells, the *souche cellulaire,* and a collateral development, the *lignes laterales.* The latter type of development, he felt, leads to a cornified squamous layer and pearl formation and thus takes no part in a further invasive process and is ultimately self destructive. According to this, only the *souche cellulaire* is affected by radiation therapy. A parallel to HN2 effect might be drawn.

Clinical results can be correlated with these morphologic observations. Of 60 patients treated with HN2 for bronchogenic carcinoma, 54 per cent showed objective evidence of improvement. When the results are analyzed according to type of tumor, 83 per cent of undifferentiated tumors, 50 per cent of squamous cell tumors, 33 per cent of adenocarcinomas and 11 per cent of epidermoid carcinomas showed such objective evidence of improvement.[24]

Of particular interest is the fact that the changes following HN2 treatment are practically identical with changes seen after ionizing irradiation. Roentgen ray changes in normal and neoplastic tissue have been amply investigated in the past. Approaches to the study of radiation effects have been made along lines similar to those along which the effects of exposure to alkyl amines are being investigated at the present time. Spear [27] has made an extensive review of the tissue culture

26. Regaud, C.: Radiophys. et radiotherap. **1**:443, 1930.
27. Spear, F. G.: Brit. J. Radiol. **8**:68, 1935.

approach. Warren [28] has studied the effects on living animals, and Stewart [29] has reviewed the cytologic changes occurring in human neoplasms after these have been exposed to roentgen radiation. Not only have the modes of study been similar but the results have been parallel. In both cases it is the nucleus which appears to suffer first and primarily. The "law of radiosensitivity" appears to apply to the alkyl amines. In both instances there is an almost instant chemical reaction within the cell, and in both there is a latent period before morphologic changes occur. Graef and co-workers [30] have recently published a study of the pathologic effects of lethal doses of HN2 as observed in animals. From their autopsy material showing involution of lymph nodes, thymus, spleen and bone marrow and necrosis of the intestinal tract they concluded that fatal roentgen irradiation produces effects in animals which are extraordinarily similar. They pointed out that even in such a small detail as the unusual HN2 resistance exhibited by chickens and pigeons there are similarities to the effect of ionizing radiation. A comparison of the two effects must be made on a superficial plane; the specific mode of action has as yet not been determined in either case.

The morphologic changes following toxic doses in normal tissue in these cases were in most respects similar to the changes found in animals.[30] We were unable to demonstrate changes in intestinal mucosa, since immediate autopsy was not possible in most cases and postmortem autolysis interfered with comparative study. One of the unusual autopsy observations might be mentioned at this point. In 2 cases death was due to overwhelming sepsis. The extent of the necrosis and the invasiveness of the infectious process were considered quite striking, particularly since both patients received full doses of penicillin for two weeks prior to death. This suggests that in addition to interfering with the cellular defense mechanism perhaps HN2 inhibits the antibody mechanism also. So-called "late deaths" of laboratory animals following mustard poisoning are frequently due to infection,[30] as are the late deaths following "total body" irradiation.[28] The growth of alpha hemolytic streptococci is inhibited when these cocci are grown in normal rat serum. Karnofsky and co-workers [31] demonstrated that there is less inhibition if the streptococci are grown in serum of mustard-treated rats. The great affinity of the mustards for a wide variety of proteins and enzymes suggests that the antibody proteins might be affected also. It is suggested that nitrogen mustard treatment may be contraindicated in the

28. Warren, S. L.: Arch. Path. **34**:443, 562, 749, 917 and 1070, 1942.

29. Stewart, F. W.: Arch. Surg. **27**:979, 1933.

30. Graef, I.; Karnofsky, D. W.; Jager, V. B.; Krichesky, B., and Smith, H. W.: Am. J. Path. **24**:1, 1948.

31. Karnofsky, D. A.; Graef, I., and Smith, H. W.: Am. J. Path. **24**:275, 1948.

presence of severe infection not only because of possible leukopenia and agranulocytosis, which can usually be avoided with careful dosage and clinical observation, but also because of its effect on the antibody mechanism.

SUMMARY

Biopsy specimens of bronchogenic carcinoma were obtained and studied microscopically before, during and after nitrogen mustard treatment in 8 cases in which eleven courses of treatment were given.

Microscopic changes following treatment were distinct in the well differentiated tumors. They consisted chiefly in giant cell formation, nuclear fragmentation, increase of the number of mitoses and production of atypical mitoses. In contrast, in undifferentiated tumors a decrease of the number of mitoses and the appearance of large areas of necrosis were noted. The cytologic picture observed after treatment was as a whole similar to that found after exposure to ionizing radiation.

In 4 additional cases, the tumor had grossly disappeared from lymph nodes and the bronchial tree, and on section of previously involved areas there was no evidence of remaining tumor, fibrosis or necrosis.

Autopsy material was studied in 8 cases. Five of the patients had died of causes other than mustard treatment, while 3 received doses of the drug which proved to be fatal.

In toxic doses HN2 caused lymphocytes to disappear from lymph nodes, with condensation of connective tissue and accumulation of plasmacytes, loss of malpighian corpuscles of the spleen with deposition of hemosiderin and slight increase of the number of plasmacytes in the red pulp. In bone marrow there was complete absence of blood-forming cells, increase in number of plasmacytes and deposition of hemosiderin. The adrenal cortex showed disappearance of lipoid substance, and Sertoli cells were the only remaining structures of the testis.

In lower doses HN2 had no effect on the morphologic appearance of any organ except for diminution of myelopoietic tissue in the bone marrow with deposition of hemosiderin there and in the spleen.

EFFECT OF NITROGEN MUSTARD IN MYCOSIS FUNGOIDES

MATTHEW BLOCK, Ph.D., M.D.*
AND
JOHN C. MURPHY, M.D.
CHICAGO

FOR SEVERAL decades roentgen therapy has been the treatment of choice for mycosis fungoides. Because the cytotoxic effect of the nitrogen mustards rather closely resembled that of roentgen radiation,[1] these drugs have been used in treating tumors of the blood-forming organs [2] and more recently in the therapy of mycosis fungoides.[3] It is our purpose to present detailed microscopic observations made before, during and after treatment with methyl-bis (β-chloroethyl) amine hydrochloride (a nitrogen mustard) in a case of mycosis fungoides.

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* Senior Research Fellow, United States Public Health Service.

From the Sections of Hematology and Dermatology, Department of Medicine, University of Chicago.

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1. (*a*) Block, M.: Unpublished material from the Metallurgy Laboratory of the Manhattan Engineering District. (*b*) Kindred, J. E.: Arch. Path. **43**:253, 1947.

2. Spurr, C. L.; Jacobson, L. O.; Smith, T. R., and Barron, E. S. G.: The Clinical Application of Methyl-Bis (B-Chloroethyl) Amine Hydrochloride to the Treatment of Lymphomas and Allied Dyscrasias, in Moulton, F. R.: Approaches to Tumor Chemotherapy, American Association for the Advancement of Science, Lancaster, Pa., Science Press Printing Company, 1947, p. 306. Goodman, L. S.; Wintrobe, M. M.; McLennan, M. T.; Dameshek, W.; Goodman, M. J., and Gilman, A.: Use of Methyl-Bis (B-Chloroethyl) Amine Hydrochloride and Tris (β-Chloroethyl) Amine Hydrochloride ("Nitrogen Mustards") in the Therapy of Hodgkin's Disease, Lymphosarcoma, Leukemia and Certain Allied and Miscellaneous Disorders, in Moulton, F. R.: Approaches to Tumor Chemotherapy, American Association for the Advancement of Science, Lancaster, Pa., Science Press Printing Company, 1947, p. 338. Karnofsky, D. A.; Burchenal, J. H.; Ormsbee, R. A.; Cornman, I., and Rhoads, C. P.: Experimental Observations on the Use of the Nitrogen Mustards in the Treatment of Neoplastic Disease, in Moulton, F. R.: Approaches to Tumor Chemotherapy, American Association for the Advancement of Science, Lancaster, Pa., Science Press Printing Company, 1947, p. 293.

3. (*a*) Post, C. F., and Lincoln, C. S.: J. Invest. Dermat. **10**:135, 1948. (*b*) Philpott, O. S.; Woodburne, A. R., and Waldriff, G. A.: J. A. M. A. **135**:631, 1947. (*c*) Henstell, H. H.; Tober, J. N., and Newman, B. A.: Blood **2**:654, 1947.

CLINICAL HISTORY [4]

A. P., a 26 year old married white woman, had been treated since the age of 13 for psoriasis, diagnosed clinically and histologically. At the age of 25 lesions developed which were characteristic of mycosis fungoides. They were treated with roentgen rays, to which they showed gradually decreasing responsiveness.

Between the sixth and twenty-ninth hospital days the patient received a total of 723 Gm. of para-aminobenzoic acid orally with no apparent change in the cutaneous or the laboratory findings, but with progressive deterioration of her general status.

Beginning on the thirtieth hospital day, nitrogen mustard (0.1 mg. per kilogram of body weight) was administered daily intravenously for four consecutive days. A dramatic clinical resolution of cutaneous lesions with melting away of tumors and healing of ulcers was apparent by the third day of treatment. This reached

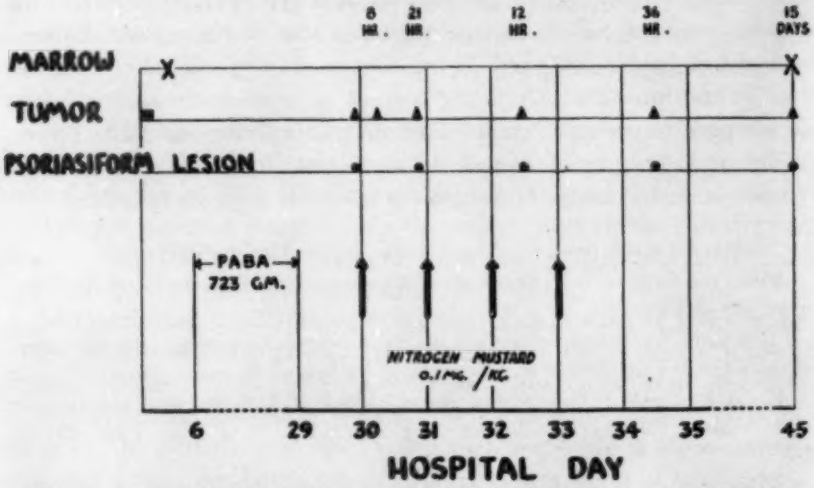

Fig. 1.—Time relation of biopsies and injection of nitrogen mustard (0.1 mg. per kilogram of body weight). *PABA* stands for para-aminobenzoic acid, administered to a total dose of 723 Gm.

a maximum on the eighth day, to continue more slowly thereafter. However, progressive leukopenia developed, beginning on the sixth day after the first injection of nitrogen mustard, and the white cell count reached a low of 375 one week later. On the seventh day after the onset of treatment the liver was tender and palpable. Scleral icterus, which appeared on the twelfth day, was followed by generalized intense jaundice. A hemorrhagic tendency developed on the thirteenth day, and thereafter the course was steadily downhill despite vigorous supportive measures, including multiple transfusions of whole blood and intravenous injection of toluidine blue. The patient died on the forty-fifth hospital day, fifteen days after the beginning of nitrogen mustard therapy.

MATERIALS AND METHODS

A tumor and the sternal marrow were submitted to biopsy prior to administration of para-aminobenzoic acid. Specimens of another tumor and a psoriasiform

4. The clinical history has been reported in detail elsewhere (Murphy, J. C., and Montgomery, H.: J. Invest. Dermat. **11**:245, 1948).

lesion were taken for biopsy immediately before and at selected intervals after starting nitrogen mustard treatment. Tissue from the same two tumors and from the sternal marrow were also obtained at autopsy (fig. 1).

The tissues were fixed in neutral formaldehyde–Zenker solution for eight hours. They were dehydrated, embedded in pyroxylin (nitrocellulose) and cut serially at 6 microns on the sliding microtome. The slides were stained with hematoxylin-eosin-azure II.

HISTOPATHOLOGIC ASPECTS OF TUMOR-LIKE LESIONS

Prior to Administration of Nitrogen Mustard.—The major part of the surface was ulcerated. Where the epidermis was intact it contained broad, flat rete ridges extending deeply into the underlying tumor tissue. Mitoses were not unusual in number or in quality. A moderate infiltrate of inflammatory cells was present in the epidermis (fig. 2 *A*). The connective tissue–epidermal boundary was intact.

The papillary layer was replaced by a densely cellular mass of tissue consisting of numerous mycosis cells, plasmacytes, eosinophilic granulocytes, occasional neutrophilic granulocytes, some of which were degenerate, and mast cells. In the mycosis cells mitoses were numerous. Many mycosis cells had large angular blocks of chromatin in their nuclei, and the cytoplasm was stained very darkly at the periphery (fig. 2 *B, a*). Others had shortened, densely staining cytoplasmic processes with a pale paranuclear area. In the latter the nuclear chromatin was extremely heavy (fig. 2 *B, b*), similar to that seen in plasmacytes (fig. 2 *C, c*).

The reticular layer of the corium was essentially similar in cellular makeup. However, the nests of plasmacytes were more numerous and larger than in the papillary layer and the mycosis cells were present in large sheets.

There had been no significant change in the histopathologic appearance of the lesion following the administration of para-aminobenzoic acid. Minor changes, such as an increase in eosinophilic granulocytes, a more delicate appearance of the mycosis cells and fewer mitoses, were so slight that they were felt to be within the range of variation to be expected in comparing two different lesions from the same patient.

Eight Hours After the First Injection of Nitrogen Mustard.—Arborization of the acanthotic rete pegs had developed. The stratum granulosum had disappeared. The epithelial papillae were wider and more swollen than prior to treatment. An increase of the amount of debris or a cessation of mitosis was not demonstrable in the epidermis.

The most marked change was in the papillary layer. An intense edema of the ground substance separated the cells. The granules of some of the eosinophilic leukocytes were strewn about, and there was some karyorrhexis of the nuclei of these cells (fig. 2 *C, b*) as well as of the nuclei of the small lymphocytes (fig. 2 *C, a*). The mycosis cells were less bizarre in appearance, and in them mitoses were definitely decreased in number. An occasional plasmacyte was seen with clumping of nuclear chromatin, but in general degeneration of plasmacytes was less frequent than that of lymphocytes and eosinophilic granulocytes. The blood vessels were dilated and filled with a basophilic precipitate.

Similar but less intense changes were seen in the reticular layer.

Twenty-One Hours After the First Injection.—Acanthosis was less marked than in the previous biopsy specimen. The cellular infiltration and edema of the epidermis were decreased in amount.

The eosinophilic granulocytes in the corium, many of which were degenerate, were greatly increased in number. The plasmacytes were decreased. There was

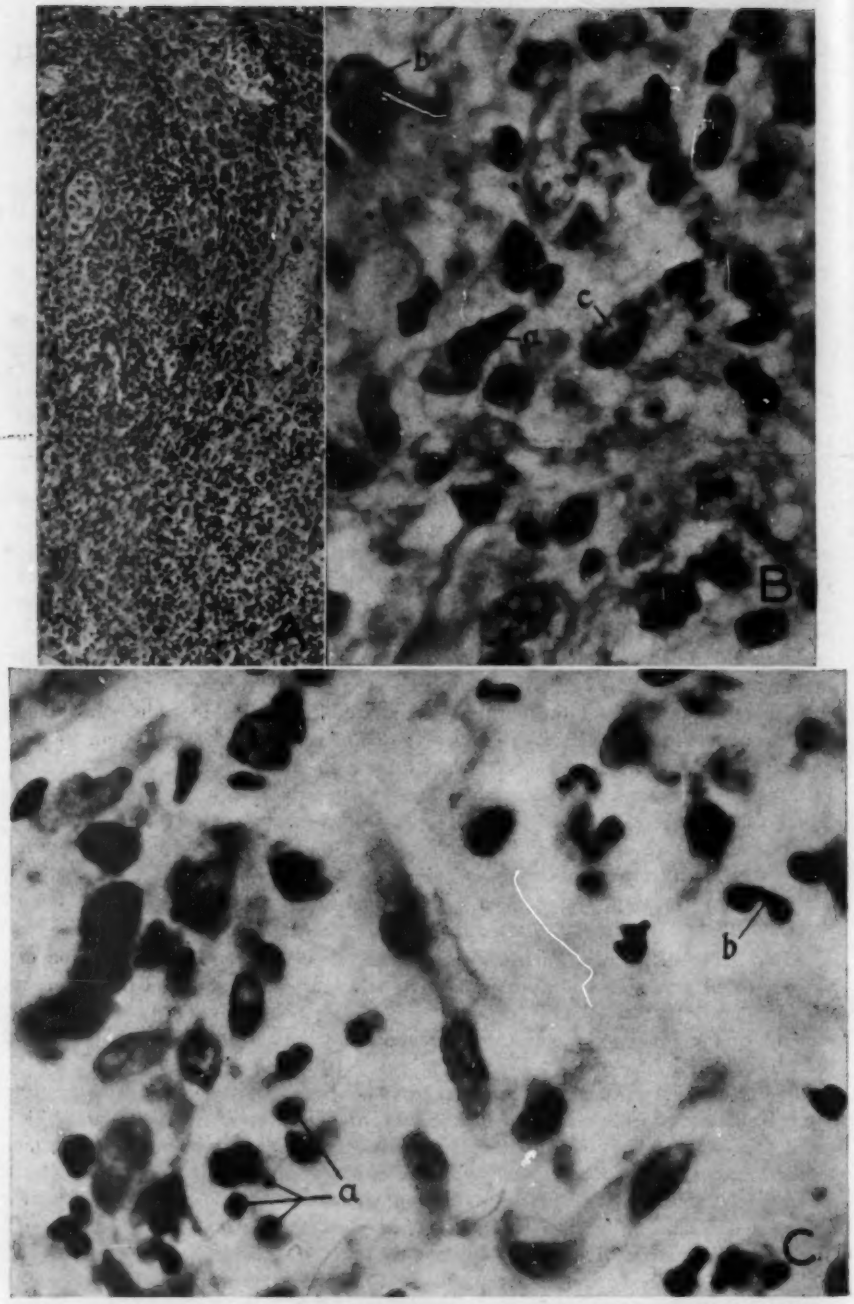

Fig. 2.—*A*, tumor-like lesion as it appeared prior to injection of nitrogen mustard. (× 104.)

B, same lesion showing a pleomorphic infiltrate in the papillary layer. Note mycosis cells, (*a*) with a heavy nucleus and (*b*) with a pale paranuclear area, and a plasmacyte (*c*). (× 980.)

C, same lesion showing edema and cellular degeneration in the papillary layer eight hours after the first injection. Note (*a*) degenerate lymphocytes and (*b*) degenerate eosinophilic granulocyte. (× 1,160.)

little edema. Degenerating cells were rare. The mycosis cells had not changed further. Except for the eosinophils, the total cellularity of the infiltrate, especially in the papillary layer, was decreased.

Twelve Hours After the Third Injection.—The epidermis was still acanthotic and without a granular layer. There was no further increase in the number of degenerate nuclei.

The cellular infiltrate of the corium was still further decreased, except for the presence of numerous small and medium-sized lymphocytes (fig. 3*A*). The mycosis cells had not changed. The plasmacytes especially were decreased in number. In mycosis cells mitosis had ceased entirely. In the reticular layer of the corium most of the previously diffuse infiltrate was now perivascular, and one could see for the first time the feltwork of collagenous fibers between the vessels.

Thirty-Six Hours After the Fourth Injection.—The involution described in the foregoing section had progressed further, and there had also been a decrease in the number of small and medium-sized lymphocytes.

Fifteen Days After the Initial Injection (Autopsy).—Acanthosis and arborization of the rete mucosum were greatly reduced (fig. 3*B*). The stratum granulosum was still absent.

The infiltrate had almost completely disappeared from the papillary layer, but occasional mycosis cells were still present. In the reticular layer the infiltrate was still quite dense and numerous mycosis cells were present, in some places in sheets. The lesion was not as pleomorphic as when seen originally, because of a relatively greater decrease in the number of free cells (eosinophilic granulocytes, plasmacytes, lymphocytes and neutrophilic granulocytes) than of fixed cells (mycosis cells). Most of the remaining infiltrate was perivascular. In mycosis cells mitoses were infrequent. Nevertheless, the lesion was still clearly recognizable as mycosis fungoides in a somewhat more acellular state as regards the free cells than existed prior to treatment (fig. 3*B*).

HISTOPATHOLOGIC ASPECTS OF THE PSORIASIFORM LESION

Prior to Administration of Nitrogen Mustard.—The epidermis was moderately acanthotic and slightly invaded by lymphocytes (fig. 3*C*).

The papillae were thicker than usual. The ascending and descending capillary loops were surrounded by small lymphocytes (fig. 3*C*). An occasional mycosis cell was demonstrable, a few of them in mitosis. The fibroblasts were not increased in number, but their nuclei were a little swollen, and the cytoplasmic processes were thicker and more heavily stained than usual. A few macrophages with phagocytosed iron were seen.

The reticular layer of the corium was normal except for a perivascular lymphocytic infiltration about the subpapillary plexus and about the vessels connecting this plexus to the deep cutaneous plexus.

Twenty-One Hours After the First Injection.—There was an increase in acanthosis and arborization of the rete mucosum. The number of lymphocytes was increased, although this was counterbalanced by an increase in degenerating lymphocytes. Mitosis had ceased entirely. Although there was no hemorrhage, the number of macrophages with phagocytosed iron granules was increased.

Twelve Hours After the Third Injection.—Parakeratosis and acanthosis were still further increased. The number of lymphocytes had not changed. Mast cells were increased in number. There was no increase of degeneration. Mitoses were completely absent.

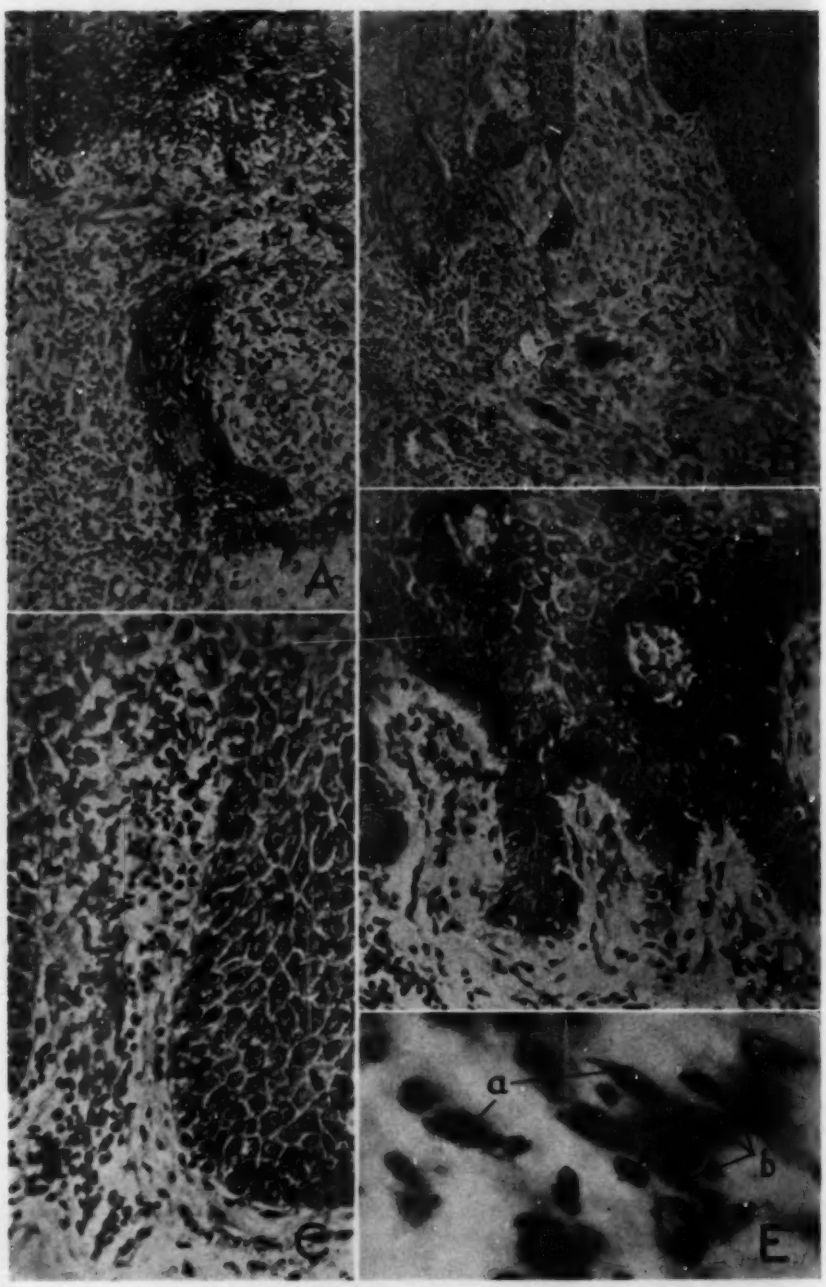

Fig. 3.—*A*, same lesion as in figure 2 *A*, showing all cells except lymphocytes decreased in number twelve hours after the third injection. (× 123.)

B, same lesion as in figure 2 *A*, showing decreased acanthosis and decreased cellular infiltrate, especially in the papillary layer, at autopsy. (× 123.)

C, flat lesion, prior to injection of nitrogen mustard, showing perivascular infiltrate. (× 245.)

D, same lesion as in *C*, showing increased arborization and only slight decrease of infiltrate at autopsy. (× 245.)

E, same lesion as in *C*, showing (*a*) increase in mast cells and (*b*) iron-containing macrophages at autopsy. (× 1,050.)

Thirty-Six Hours After the Fourth Injection.—The acanthosis and parakeratosis were a little decreased. There was less lymphocytic infiltration, and what was present was clearly perivascular. Mitoses were present but decreased in number.

Fifteen Days After Initial Injection (Autopsy).—An abnormal amount of acanthosis was still demonstrable (fig. 3*D*). The amount of infiltrate was but slightly decreased as compared with that observed prior to treatment (compare fig. 3C and *D*). Mast cells (fig. 3*E*, *a*) and iron-containing macrophages (fig. 3*E*, *b*) were more numerous than before. There were numerous hemorrhages about the subpapillary plexus and about the vessels in the superficial portion of the reticular layer of the corium.

HISTOPATHOLOGIC CHANGES OBSERVED IN OTHER ORGANS

The pretreatment marrow was rather hypercellular. There was myelocytic hyperplasia with a mild left shift and an increase in cells of the plasmacytic series. On smear the megakaryocytes and the thrombocytes were normal.

At autopsy the marrow was atrophic—about one-fourth as cellular as it was prior to treatment. Most of the hypocellularity was due to the disappearance of granulopoietic and erythropoietic cells as well as of megakaryocytes. As a result, cells of the plasmacytic series and reticular cells were much more prominent. A great deal of the marrow was occupied by a gelatinous precipitate and dilated vessels.

At autopsy all the viscera, as well as the skin, were the site of numerous hemorrhages. In the liver many of the parenchymatous cells were necrotic, perhaps as a result of pressure from the numerous extravasated red cells. There was no evidence of lymphoma in any of the internal organs.

COMMENT

The effect of nitrogen mustards on tissues is similar to that of ionizing radiations. In the normal animal given median lethal to lethal doses of nitrogen mustard the successive changes are as follows: First there are cessation of mitosis and degeneration of susceptible cells (small lymphocytes, myelocytes, erythroblasts and megakaryocytes). The major part of the degeneration of susceptible cells occurs only in the hemopoietic tissues, within eighteen hours after injection, not in the peripheral blood. The outstretched fixed cells (fibroblasts, macrophages and reticular cells) as well as plasmacytes are extremely resistant to the destructive action of the mustards. The second phase is phagocytosis of debris. The third, or atrophic, phase lasts several days and corresponds to a period of aplasia of the susceptible cells and reduction of the number of mitoses. Regeneration ensues as a result of heteroplastic formation of the susceptible free cells from the reticular cells and homoplastically by mitotic proliferation of the few residual susceptible cells.[1] There is no evidence to support the thesis that the nitrogen mustards exert their effect primarily on immature or rapidly dividing cells.

Henstell and co-workers,[3c] after serial observations on well controlled biopsy material, described a decrease in density of the infiltrate in

mycosis fungoides. They concluded that the drug exerts its effect primarily on "reticulo-endothelial cells." In their illustrations and descriptions, degeneration of such cells is not apparent. In any case it is rather doubtful that the mycosis cell belongs to the "reticulo-endothelial system" as originally interpreted by Aschoff.[5]

In general, our results confirm those of Henstell and co-workers[6c] so far as the decrease in the infiltrate is concerned. However, in our material there was little evidence of any marked effect on the mycosis cells themselves except as regards the cessation of mitosis. There was always some degeneration in the mycosis cells prior to treatment, and there was no significant increase of this degeneration following treatment. Judging from the experimental work demonstrating the resistance of fixed cells, such as fibroblasts and reticular cells, to nitrogen mustard,[6] one would hardly expect any marked destructive effect on the mycosis cells, which also are fixed cells. Inhibition of mitosis, coupled with spontaneous degeneration, causes a decrease in the number of mycosis cells. However, it is difficult to determine whether the combination of these two factors alone adequately accounts for the decrease in number of mycosis cells.

The histopathologic effects of nitrogen mustard in Hodgkin's disease and lymphosarcoma, which are related to mycosis fungoides, have been studied.[6a] Parallel with our experience, in those diseases it was impossible to account for the decrease in size of the tumorous organs by the amount of cellular degeneration seen. As a result of numerous follow-up biopsies, it was emphasized that nitrogen mustard could destroy only those cells in tumors which it also destroys in normal tissue; in addition, inhibition of mitosis was described . At no time (again in agreement with the results of our study) was it possible to demonstrate a change in the basic structural pattern of any lesion at biopsy.[6a]

Although our patient died before an exacerbation occurred, from other reports on the clinical course of mycosis fungoides following injection of nitrogen mustard[3] there is little doubt that such an exacerbation, manifested by a proliferation of the residual mycosis cells and a reappearance of the free cells, would have occurred, in a manner analogous to that seen in Hodgkin's disease.[6a]

Because of the similarity of the histopathologic effects of nitrogen mustard and roentgen radiation, it is interesting to compare the effects of the two on the lesions of mycosis fungoides. Unfortunately, there are few controlled observations of the histopathologic effects of the

5. Aschoff, L.: Ergebn. d. inn. Med. u. Kinderh. **26**:1, 1924.

6. (*a*) Block, M.; Spurr, C. L.; Jacobson, L. O., and Smith, T. R.: Am. J. Clin. Path. **18**:671, 1948. (*b*) Block.[1a] (*c*) Kindred.[1b]

irradiation of the lesions of mycosis fungoides. The disease is notoriously pleomorphic in its histologic manifestations, and the individual lesions tend to change from time to time. Consequently, unless a pretreatment biopsy is made immediately prior to the start of therapy to serve as a basis for comparison of the post-treatment biopsies, it is impossible to evaluate the effects of treatment.

In the literature there are two articles on the histopathologic effect of roentgen rays on mycosis fungoides, one by Herxheimer and Hübner [7] and the other by Pautrier.[8] They have described the disappearance of the infiltrate after local irradiation and have expressed the belief that there was a specific effect on the mycosis cell although they did not substantiate this concept except by theorizing that the mycosis cell is embryonic in nature and consequently radiosensitive. Lubarsch and Wätjen, in their review [9] of histopathologic observations of radiation therapy, question the validity of this explanation. The most recent and by far the most exhaustive study of the histopathologic effects of radiation, edited by Bloom,[10] has refuted this time-honored but otherwise poorly substantiated theory.

From a review of the reports on the histopathologic effects of nitrogen mustard and roentgen rays on the lesions of the skin of patients with mycosis fungoides, as well as from our own study, only two facts are demonstrable, namely, that the cellularity of the tumor-like lesion decreases and that at no time is a lesion healed microscopically. To this one may add that a destructive effect on the lymphocytes, the granulocytes and, to a much less extent, the plasmacytes and a decrease in the number of mitoses in mycosis cells are demonstrable. As in a previous study on tumors of the hemopoietic organs treated with nitrogen mustard,[9a] the amount of cellular destruction objectively demonstrated by a comparison of pretreatment and post-treatment biopsy specimens in cases of mycosis fungoides does not seem adequate to account for the clinical improvement noted. There is no more evidence to support the presence of a specific effect on embryonic cells or on reticuloendothelial cells after treatment with nitrogen mustard than after irradiation.

No severe hemorrhagic manifestations have been reported following a single course of nitrogen mustard therapy in mycosis fungoides

7. Herxheimer, K., and Hübner, H.: Arch. f. Dermat. u. Syph. **84**:241, 1907.

8. Pautrier, L. M.: Strahlentherapie **6**:257, 1915.

9. Lubarsch, O., and Wätjen, J.: Allgemeine und spezielle pathologische Histologie der Strahlenwirkung, in Lazarus, P.: Handbuch der gesamten Strahlenheilkunde, Munich, J. F. Bergmann, 1928, vol. 1, p. 304.

10. Bloom, W.: The Histopathology of Irradiation from External and Internal Sources, National Nuclear Energy Series (Manhattan Project Technical Section), New York, McGraw-Hill Book Co., Inc., 1948, vol. 22 I.

with the dosage of 0.1 mg. per kilogram on four successive days.[3] The gelatinous aplasia of the marrow at autopsy in the case presented was far greater than had been noted following injection of nitrogen mustard at the standard dose level [6a] and corresponded in degree to that seen at higher dosage levels in human beings and animals and in terminal stages of Hodgkin's disease.[6] Consequently, one cannot help but wonder whether the combination of para-aminobenzoic acid and nitrogen mustard does not have a more depressing action on the marrow than does nitrogen mustard alone. The increase in mast cells noted especially in the autopsy specimen may also have had some relation to the hemorrhagic tendency, because the mast cells have been related to the increase of a heparin-like substance in the blood after irradiation and after treatment with nitrogen mustard.[11]

SUMMARY AND CONCLUSIONS

Within a few hours after injection of nitrogen mustard there is evidence of a degeneration of granulocytes, small lymphocytes and, to a much less extent, of plasma cells and mycosis cells. The number of mitoses in mycosis cells is greatly decreased, and in some stages mitosis is inhibited.

Objective histopathologic evidence fails to support the concept of a destructive effect of nitrogen mustard on reticuloendothelial cells or cells of embryonic nature.

Histopathologic study of the lesions after treatment has failed to account for the marked change in the lesions seen clinically.

Histologic observation failed to demonstrate a complete cure of any lesion of mycosis fungoides at any time after treatment with nitrogen mustard.

At all intervals at which a specimen was obtained for biopsy it was always possible to identify the lesion as mycosis fungoides. Treatment had produced a comparatively acellular lesion without in any way modifying the fundamental nature of the disease.

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11. Jorpes, J. E.: Heparin in the Treatment of Thrombosis, New York, Oxford University Press, 1946.

BRONCHIAL ADENOMA PRODUCING AN "ALVEOLAR CELL CARCINOMA" PATTERN

J. H. CHEEK, M.D.
AND
E. E. MUIRHEAD, M.D.
DALLAS, TEXAS

IN RECENT years "alveolar cell carcinoma," [1] "alveolar cell tumor," [2] "pulmonary adenomatosis" [3] and "lobar carcinoma" [4] have been observed in man and described. A comparable process has been observed in the disease of sheep known as jaagsiekte.[5] In these conditions the alveolar septums become partially or completely lined by cuboidal to columnar epithelium, usually of a single layer, but at times stratified in focal areas. Papillary projections into the alveolar spaces can frequently be demonstrated.

The origin of such processes has been much discussed and remains even now an unsettled matter. A recognized change in chronic pneumonitis is a cuboidal lining of the alveolar spaces. The histologic resemblance of the infectious disease of sheep, jaagsiekte, to "pulmonary adenomatosis" of man is striking, though all efforts to prove that the latter is of an infectious origin have been unsuccessful. Some have believed that perhaps these two diseases are identical and are not unlike the so-called "alveolar cell carcinoma" or "alveolar cell tumor." [3] Herbut [6] expressed the opinion that the basal layer of cells in bronchi or bronchioles breaks through the alveolar walls and extends along the lining of the alveolar spaces as cuboidal and low columnar epithelium. He could find no evidence that these cells had their origin from cells lining the alveolar spaces. Neubuerger and associates [7] expressed the belief that the cells giving the "alveolar cell" picture have their origin from the cells which line the alveolar spaces. Because

From the Department of Surgical Pathology, Baylor University Hospital.

1. Ikeda, K.: Am. J. Clin. Path. **15**:50, 1945.

2. (*a*) Neubuerger, K. T.: J. Thoracic Surg. **10**:557, 1941. (*b*) Herbut, P. A.: Am. J. Path. **20**:911, 1944.

3. Simon, M. A.: Am. J. Path. **23**:413, 1947.

4. Silverman, G., and Angrist, A.: Arch. Int. Med. **8**:369, 1948.

5. Dungal, N.: Proc. Roy. Soc. Med. **31**:497, 1938.

6. Herbut, P. A.: Arch. Path. **41**:175, 1946. Herbut.[2b]

7. Neubuerger, K. T., and Geever, E. F.; Arch. Path. **33**:551, 1942. Geever, E. F.; Carter, H. R.; Neubuerger, K. T., and Schmidt, E. A.: Radiology **44**:106, 1945. Neubuerger.[2a]

these cells were considered independent of the bronchial lining, and because a controversy concerning them existed, these workers preferred the term "alveolar cell tumor" rather than "carcinoma."

There has also been considerable discussion concerning the histogenesis of the "bronchial adenoma," apparently because of its variable histologic picture. Graham and Womack [8] found that different areas revealed different patterns within the same tumor. The close resemblance of some adenomas to fetal lung suggested that these tumors could have arisen from endobronchial buds which had failed to develop. The origin of bronchial adenoma from peribronchial mucous glands or from the basal cell layer of the bronchial lining has been thought to be the basis for the pattern an adenoma may show.[9] Thus, according to this view, adenoma arising from the basal layer of cells may give rise to tumors composed of small cells, with scanty cytoplasm and small, round, dark-staining nuclei, which tend to arrange themselves in clusters or cords, and adenoma from glands may show a tumor with varying glandular arrangement and composed of large polygonal or columnar cells with wide zones of pale, pink-staining, finely granular cytoplasm and with fairly large vesicular to dark-staining nuclei. Attention has been repeatedly directed to the close resemblance of adenoma to mixed tumors of the salivary glands, carcinoid of the appendix, basal cell carcinoma of the skin, so-called cylindroma and even to islet cell tumors of the pancreas and parathyroid adenoma. In fact, bronchial adenoma has been at times classified according to these similarities. Graham and Womack [8] accounted for the mixed tumor appearance on the basis that the stroma may become dominated by cells of mesoblastic origin giving rise to connective tissue, muscle, cartilage, fat and bone. Holly [10] used the term "carcinoid" because the cells of the tumor, the growth characteristics and the cellular arrangement seem to be identical with those of carcinoid of the appendix. Stout [11] expressed the belief that oncocytes, special cells found in the wall of bronchial ducts and glands, may be the origin, since he has been able to demonstrate them in some of the adenomas.

We wish to report 2 representative cases of bronchial adenoma in which the histologic picture at the advancing edge of the tumor or elsewhere in the lung has been identical with that seen in pulmonary adenomatosis or the so-called alveolar cell carcinoma or tumor. One of these cases (no. 2) was revealed in a surgical specimen, and the other, at autopsy.

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8. (*a*) Graham, E. A., and Womack, N. A.: J. Thoracic Surg. **14**:106, 1945. (*b*) Womack, N. A., and Graham, E. A.: Arch. Path. **26**:165, 1938.

9. (*a*) Fried, B. M.: Arch. Int. Med. **79**:291, 1947. (*b*) Sano, M. E., and Meade, R.: Arch. Path. **43**:235, 1947.

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11. Stout, A. P.: Arch. Path. **35**:503, 1943.

REPORT OF CASES

CASE 1.—A 44 year old white woman entered the Baylor University Hospital, Oct. 29, 1947, with shortness of breath and coughing of blood for a period of three months. Previously she had been in good health. She had lost 8 to 10 pounds

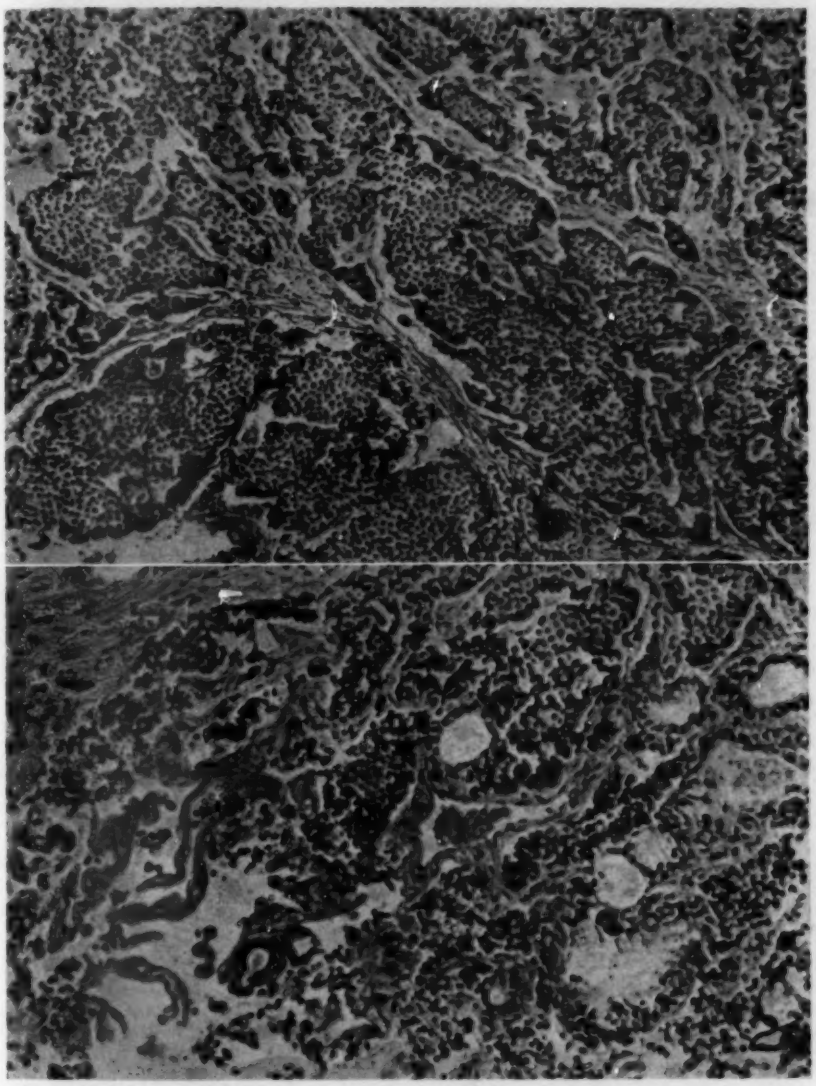

Fig. 1 (case 1).—A typical bronchial adenoma with the so-called carcinoid pattern in the wall of the larger bronchus. Two mucus-filled spaces can be seen in the left upper corner.

Fig. 2 (case 1).—The edge of the tumor in figure 1 showing a mixture of carcinoid and mucus-secreting glandular elements with a greater amount of mucus-secreting glandular structures present. Beginning of lining of alveolar walls can be detected.

(3.5 to 4.5 Kg.) in weight and had fever (temperature, 103 F.) with no chills. Deep inspiration caused cough, and this exaggerated the dyspnea. There was no family history of tuberculosis or of tuberculous contacts. The patient was well nourished and well developed. The blood pressure was 110 systolic and 80 diastolic. The pulse rate was 145; the respiratory rate, 22. She was more comfortable lying flat in bed, but even so her respiration was labored. No cyanosis was noted. There was a deep, nonproductive cough. The chest expanded equally in all directions. Flatness was noted over the upper part of the chest, both anteriorly and posteriorly, and the breath sounds were high pitched over these areas. There was increased dyspnea with coughing when the patient tried to sit up in bed.

A roentgenogram of the chest revealed extensive miliary infiltrations, and it was thought that there was an enlargement of the hilar lymph nodes.

Course in Hospital.—Skin tests with coccidiodin and histoplasmin gave negative results. The temperature varied from 98.6 to 100 F.; the pulse rate, from 110 to 158, and respirations occurred at a rate of between 18 and 54 per minute. Numerous examinations of sputum revealed no acid-fast bacilli. Oxygen therapy was started and intramuscular injections of streptomycin were given, but the patient's condition showed little change. She gradually grew worse and died on the fifth hospital day. Pneumonitis, possibly tuberculous, and carcinomatosis were the clinical impressions.

Autopsy.—The lungs were shrunken and firm in consistency. The surfaces were finely nodular, with the nodules varying in size from 1 to 5 mm. and of a grayish yellow color. Throughout both lungs were scattered nodules of a similar size and color. Small nodules of a similar type were noted on the surface of the liver and within the hepatic parenchyma. Nodules were also found within the adrenal glands, the parietal pericardium and mediastinal lymph nodes. At the bifurcation of the bronchus to the lower lobe of the right lung was a grayish red polypoid mass, measuring 1 by 0.5 cm., which was friable and which almost completely obstructed the bronchus. It extended for a few millimeters into both limbs of the bifurcation.

The tumor within the bronchus to the lower lobe of the right lung was composed of small, dark-staining cells of uniform size. The histologic picture was that of the carcinoid tumor (fig. 1 and 2). Other areas of the tumor were composed of tall, columnar, mucus-secreting epithelium lining spaces containing mucus (fig. 3). At one margin of the tumor was a transition from the carcinoid picture to the alveolar cell arrangement. Here again the cells were tall, columnar, with oval to round vesicular nuclei and pale, pink-staining cytoplasm which in many instances showed secretory vacuoles. These cells were growing into and lining the alveolar spaces. Sections from the areas of metastases (fig. 4) showed similar tumor cells lining alveolar spaces; these spaces often contained a mucinous material.

CASE 2.—M. M., a 53 year old woman, was admitted to the hospital with the history that a routine roentgenogram of the chest in March 1947 revealed a "spot" in the upper lobe of the right lung. The patient continued to perform her normal duties and continued to have roentgen examinations of the chest every three months. In July 1948 there was noted a slight increase in the size of the lesion, and on the basis of this finding an operation was recommended. During this period of time she had been asymptomatic. Her past history was noncontributory except for serious pneumonia at the age of 6 and hysterectomy in 1943. She was a well nourished woman with a blood pressure reading of 120 systolic and 78 diastolic; small, firm, nontender nodes were palpated in the left cervical region. Roentgenograms revealed a circumscribed lesion near the periphery of the base of the upper lobe of the right lung. On Aug. 2, 1948 (sixteen months after the discovery of the lesion) thoracotomy was done. A tumor was found within the anterior segment of the upper lobe of the right lung, measuring roughly 2 cm. in diameter. Puckering

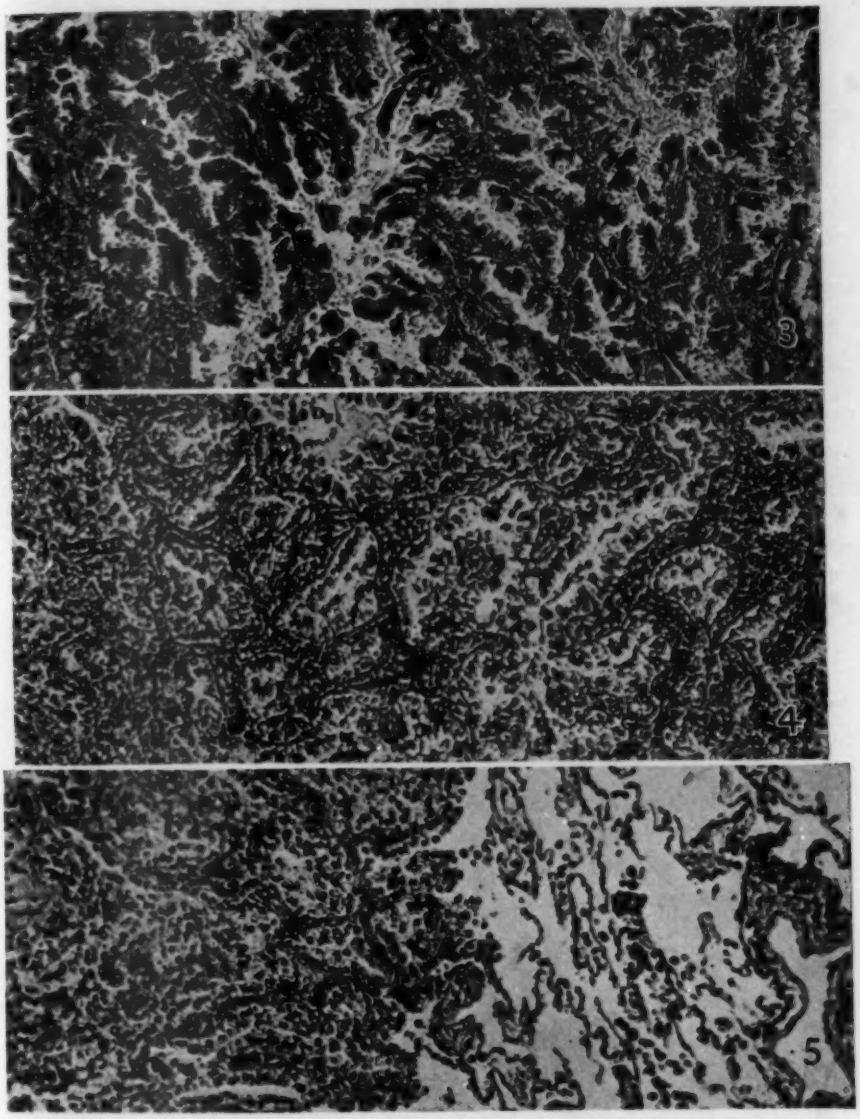

Fig. 3. (case 2).—The glandular, mucus-secreting make-up of the so-called "alveolar cell" carcinoma at its center is demonstrated.

Fig. 4 (case 1).—Papilliferous structure of a metastatic nodule of the opposite lung. Lining of alveolar walls with columnar cells of a mucus-secreting type is evident. This pattern was observed not only as a transition from the adenoma but elsewhere in both lungs.

Fig. 5 (case 2).—The advancing edge of the tumor. Utilization of the lung structure as a scaffold for growth and lining of the alveolar walls are demonstrated.

of the overlying pleural surface was noted. There were no enlarged hilar nodes. The tumor was excised widely, and the postoperative course was satisfactory.

Grossly, the lesion on section was fairly well circumscribed and measured 2 cm. in diameter. It was composed of a moderately dense, mottled, grayish yellow tissue in which were multiple minute dark areas considered to be pigment.

Microscopically (fig. 4), the tumor was composed of cuboidal to tall columnar or polyhedral cells, which formed glandular structures at the center. These cells had fairly large oval to round vesicular nuclei and pale, pink-staining, finely granular cytoplasm. In some areas a piling up or pseudostratification was noted, as were papillary projections of cells extending into some of the alveolar spaces. At the periphery (fig. 5) these cells were growing out into and lining the alveolar spaces. In some areas one side of the alveolar septum was lined by these cells while the opposite side showed no cells.

COMMENT

It seems that the so-called alveolar cell pattern is not pathognomonic of any one process, since it can be seen in infectious processes, in relatively benign tumors and in cancers, as well as in the metastatic lesions of the lungs. It has been previously pointed out that metastatic adenocarcinoma can produce an alveolar cell pattern in the lung.[6] We have seen this pattern in adenocarcinoma of the breast, the stomach, the prostate, the kidney and the pancreas and in one transitional cell carcinoma of the bladder. It is of further interest that in certain cases carcinoma of the lung has in one area a definite squamous cell and in another area a clearcut alveolar cell carcinoma pattern. In our cases the alveolar cell pattern was well demonstrated, with cuboidal and columnar cells growing out into and lining the alveolar spaces in a uniform manner and, in focal areas, producing papillary projections.

Bronchial adenoma occurs more frequently in women and is usually described as observed in the third and fourth decades of life, whereas bronchiogenic carcinoma occurs in the fifth and sixth decades and is more prevalent in men. The question of cancer with regard to bronchial adenoma continues to be a serious one. Some feel that all of these tumors should be considered cancerous but at the same time should be separated from bronchiogenic carcinoma.[12] Cases in which metastases occurred are reported in support of this view. Others consider the tumor relatively benign and only occasionally cancerous.[13] In our experience bronchial adenoma is locally invasive and may produce metastases in nodes, the opposite lung and elsewhere.

The morbidity, the symptoms and the signs associated with bronchial adenoma usually are due to bronchial obstruction and to secondary

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13. Allen, I. V.: Canad. M. A. J. **55**:498, 1946. Fried.[9a] Sano and Meade.[9b] Holly.[10]

inflammatory processes, which may suggest some other pulmonary disease. We believe that in case 2 the tumor was obviously a relatively benign bronchial adenoma, because of its long course and lack of cancerous change. Lack of symptoms referable to it can probably be accounted for on the basis of its peripheral location with subsequent lack of bronchial obstruction and secondary inflammatory changes. The tumor represented a bronchial adenoma the origin of which could not be identified but the cells of which suggest that it originated from peribronchial glands. It was composed of cuboidal to columnar cells, which were growing out into and lining the alveolar spaces, giving a definite "alveolar cell carcinoma" pattern. In case 1 a bronchial adenoma with the typical carcinoid picture was demonstrated. At its periphery was a transition into a tumor composed of taller cells lining alveolar spaces and producing the "alveolar cell carcinoma" pattern. The tumor had produced widespread pulmonary extensions and metastases to the hilar nodes, the pericardium, the liver and the adrenal glands, producing in these sites a pattern like that of the original tumor.

SUMMARY

The histologic pictures of pulmonary adenomatosis and "alveolar cell carcinoma" have been discussed, after a brief review of the ideas of the origins of these tumors. The usual arrangement or picture of bronchial adenoma and its origin have been discussed. Two cases of bronchial adenoma have been reported in which the tumor produced an "alveolar cell carcinoma" or adenomatosis pattern. Both tumors were of low grade histologic appearance. One was locally invasive only; the other was locally invasive and produced widespread pulmonary extensions and distant metastases.

EXPERIMENTAL ATHEROSCLEROSIS

X. The Effect of Desoxycorticosterone Acetate on the Cholesterol Content of the Blood, the Aorta and the Liver of the Rabbit

MAURICE BRUGER, M.D.
AND
BERTRAND E. LOWENSTEIN, M.D.
NEW YORK

HYPERCHOLESTEREMIA and atherosclerosis develop readily in the cholesterol-fed rabbit,[1] but their severity can be affected by a number of factors. Since hypercholesteremia and atherosclerosis represent abnormalities of metabolism, it is logical to expect that the endocrine system (one of the major governors of metabolism) should exert considerable influence on these effects, and this expectation has, in fact, been borne out experimentally. Thus, the administration of either an inorganic iodide or thyroid gland substance inhibits the deposition of cholesterol in the arterial tree,[2] although injection of anterior pituitary thyrotropic extract not only fails to inhibit but actually enhances the tendency toward atherosclerosis.[3] The steroid hormones also are effective. Testosterone propionate and estradiol dipropionate inhibit hypercholesteremia and atherosclerosis in the female rabbit but have no protective action either in the male [4] or the castrate female.[5] In birds blood lipids are known to rise after the

From the Medical Research Laboratory, Department of Medicine, Post-Graduate Medical School, New York University–Bellevue Medical Center.

1. Anitschkow, N.: Beitr. z. path. Anat. u. z. allg. Path. **56**:379, 1913. Wacker, L., and Hueck, W.: München. med. Wchnschr. **60**:2097, 1913; Arch. f. exper. Path. u. Pharmakol. **74**:416, 1913. Duff, G. L.: Arch. Path. **20**:81, 1935.

2. Leary, T.: Arch. Path. **21**:459, 1936; J. A. M. A. **105**:475, 1935. Bruger, M., and Rosenkrantz, J. A.: J. Clin. Endocrinol. **2**:176, 1942. Murata, M., and Kataoka, S.: Verhandl. d. japan. path. Gesellsch. **7**:27, 1917. Liebig, H.: Arch. f. exper. Path. u. Pharmakol. **159**:265, 1930; **175**:409, 1934. Seel, H., and Creuzberg, G.: ibid. **161**:674, 1931. Turner, K. B.: J. Exper. Med. **58**:115, 1933. Friedland, I. B.: Ztschr. f. d. ges. exper. Med. **87**:683, 1933. Page, I. H., and Bernhard, W. G.: Arch. Path. **19**:530, 1935. Turner, K. B., and Khayat, G. B.: J. Exper. Med. **58**:127, 1933.

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4. Ludden, J. B.; Bruger, M., and Wright, I. S.: Arch. Path. **33**:58, 1942.

5. Bruger, M.; Wright, I. S., and Wiland, J.: Arch. Path. **36**:612, 1943.

administration of estrogens,[5] although testosterone, progesterone, androstenedione and desoxycorticosterone are all without effect.[7]

The interrelationship between the adrenal cortex and cholesterol metabolism has also been studied. Thus, Maranon and Collazo [8] noted that in the presence of a hypofunctioning adrenal cortex, hypocholesteremia developed, while increased adrenal activity produced hypercholesteremia. These observers also reported that the administration of adrenal cortex extract to either man or dog increased the blood cholesterol. Fiandacca and Capizzi [9] used three fractions of whole adrenal cortex and found that one fraction corrected hypocholesteremia, another lowered the blood cholesterol, while the third had no influence on the blood level of this lipid. Villela [10] claimed that the administration of desoxycorticosterone lowered the plasma cholesterol in normal guinea pigs.

More recently, Long and his collaborators [11] have shown that if the adrenal cortex is stimulated with anterior pituitary adrenotropic extract, epinephrine or nociceptive stimuli, the stimulation is followed by an impressive decrease in the cholesterol content of the adrenal gland. Long postulated that this change may represent conversion of cholesterol into the characteristic adrenal cortical steroids.

On the other hand, Baumann and Holly [12] studied the effect of unilateral and bilateral adrenalectomy on the blood cholesterol and lipoid phosphorus of rabbits and observed no significant changes. Randles and Knudson [13] found that the removal of the adrenal glands of rats did not alter the blood cholesterol level, while Entenman and associates,[7] working with chickens, failed to raise the blood cholesterol, total fatty acid or phospholipid values by injecting desoxycorticosterone acetate. Harrop and his co-workers [14] were unable to influence the blood cholesterol of either man or dog by injecting adrenal

6. Lorenz, F. W.; Chaikoff, I. L., and Entenman, C.: J. Biol. Chem. **126**:763, 1938. Zondek, B., and Marx, L.: Arch. internat. de pharmacodyn. et de thérap. **61**:77, 1939. Landauer, W.; Pfeiffer, C. A.; Gardner, W. U., and Man, E. B.: Proc. Soc. Exper. Biol. & Med. **41**:80, 1939.

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8. Maranon, G., and Collazo, S. A.: Rev. franç. d'endocrinol. **13**:1, 1935.

9. Fiandacca, S., and Capizzi, I.: Endokrinologie **14**:316, 1934.

10. Villela, O.: Hospital **19**:41, 1941.

11. Long, C. N. H.: Federation Proc. **6**:461, 1947.

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13. Randles, F. S., and Knudson, A.: J. Biol. Chem. **76**:89, 1928.

14. Harrop, G. A., Jr.; Swingle, W. W., and Pfiffner, J. J.: J. Biol. Chem. 92: lvi, 1931.

cortex extract. Furthermore, Raab and associates [15] were unable to influence the course of experimental atherosclerosis in the rabbit by either adrenalectomy or by the administration of either adrenal cortex extract or anterior pituitary adrenotropic extract.

While the bile acids are not hormones, they possess a steroid structure, and it therefore seems worth while to mention their effects

TABLE 1.—*Cholesterol Contents of Blood, Aorta and Liver of 24 Young Female Rabbits Fed 1.0 Gm. of Cholesterol Three Times per Week for Twelve Weeks*

	Whole Blood Cholesterol (Mg. per 100 Cc.)														Liver Cholesterol, Mg. per 100 Gm. Wet Wt.	Aorta Cholesterol, Mg. per 100 Gm. Dry Wt.
	Total Cholesterol							Ester (per Cent of Total)								
Rabbit	0 Wk.	2 Wk.	4 Wk.	6 Wk.	8 Wk.	10 Wk.	12 Wk.	0 Wk.	2 Wk.	4 Wk.	6 Wk.	8 Wk.	10 Wk.	12 Wk.		
14	80	610	690	850	940	940	670	13	53	40	55	58	48	52	2,540	3,470
15	170	250	250	200	820	1,340	1,070	29	44	42	54	52	56	82	1,890	810
16	100	210	410	190	550	890	690	19	24	28	48	48	47	59	2,400	690
17	130	280	290	610	870	1,010	...	17	33	41	52	54	47	..	980	4,370
18	90	120	100	280	710	1,250	1,010	13	30	28	62	48	51	56	3,710	2,300
19	96	260	190	120	390	1,250	1,170	20	23	11	23	57	70	68	2,840	1,610
20	100	210	210	490	640	940	940	18	22	12	44	58	67	51	2,350	1,530
21	130	150	130	450	750	850	680	17	31	31	55	57	58	61	1,510	3,480
22	100	180	340	360	660	960	870	..	..	..	..	..	..	..	700	2,840
23	130	300	250	220	250	240	600	..	..	..	..	..	..	..	450	660
24	110	380	560	940	820	890	630	21	54	46	51	50	48	40	940	9,020
25	110	220	390	330	420	660	660	17	39	47	23	42	50	30	1,010	1,650
26	140	290	500	600	680	770	530	25	37	44	40	41	42	41	640	2,540
27	99	210	350	420	750	1,100	1,100	34	54	44	47	41	41	51	510	3,280
28	130	340	180	150	280	260	270	22	55	53	47	50	36	28	440	980
29	99	370	630	670	630	990	1,140	25	41	62	38	50	47	36	720	3,540
30	85	260	270	420	710	550	...	22	49	66	47	51	29	..	1,120	1,100
70	64	190	520	730	800	1,100	850	9	39	64	63	70	55	44	1,960	5,220
71	64	200	68	160	250	200	280	14	32	31	59	44	30	24	960	510
72	92	300	640	850	780	940	900	11	55	57	63	56	52	41	1,700	6,000
73	69	180	480	510	580	730	750	14	21	71	69	55	57	50	1,250	6,010
74	66	200	210	420	310	400	350	13	38	53	71	45	43	50	1,010	1,550
75	64	630	800	940	1,010	900	800	9	57	64	71	19	60	35	2,080	3,320
76	63	660	830	820	900	1,140	1,070	17	55	57	57	19	45	43	2,470	5,210
Average	99	300	390	490	650	850	790	18	41	45	52	48	49	47	1,510	3,050

here. Simultaneous feeding of bile salts and cholesterol to mice greatly augments the amount of cholesterol deposited in the liver over that found if only cholesterol is fed.[16] In work published from this laboratory, it was shown that the cholesterol content of the blood and the aorta of the cholesterol-fed rabbit could be greatly increased if cholic

15. Raab, W.; Wachstein, M., and Straubb, S.: Ztschr. f. d. ges. exper. Med. **102**:212, 1937.

16. Hummel, R.: Ztschr. f. physiol. Chem. **185**:105, 1929. Loeffler, K.: ibid. **178**:186, 1928.

acid or glycocholic acid was added to the dietary regimen.[17] This effect was ascribed to the role of bile acids in favoring absorption of cholesterol from the gastrointestinal tract and not to the related chemical configuration.

In view of the fact that these studies do not permit definite conclusions regarding the influence of adrenal cortical steroids on experimental atherosclerosis, the present experiment was undertaken to ascertain the effect of desoxycorticosterone acetate on the blood cholesterol levels and on the cholesterol content of the aorta and the liver of the young female rabbit fed cholesterol.

TABLE 2.—*Effect of 1.5 to 2.5 Mg. of Desoxycorticosterone Acetate Injected Triweekly on the Cholesterol Content of Blood, Aorta and Liver of 26 Young Female Rabbits Fed 1.0 Gm. of Cholesterol Three Times per Week for Twelve Weeks*

	Whole Blood Cholesterol (Mg. per 100 Cc.)														Liver Cholesterol, Mg. per 100 Gm. Wet Wt.	Aorta Cholesterol, Mg. per 100 Gm. Dry Wt.
	Total Cholesterol							Ester (per Cent of Total)								
Rabbit	0 Wk.	2 Wk.	4 Wk.	6 Wk.	8 Wk.	10 Wk.	12 Wk.	0 Wk.	2 Wk.	4 Wk.	6 Wk.	8 Wk.	10 Wk.	12 Wk.		
1	81	390	410	580	500	590	430	26	45	62	47	41	51	48	1,550	7,210
3	100	680	770	1,040	1,010	780	770	22	47	52	55	61	51	56	2,160	5,860
4	92	530	550	540	670	...	...	24	45	56	..	54	..	..	910	760
8	96	320	590	730	1,500	1,440	...	19	49	48	66	67	61	..	3,530	1,790
9	110	290	490	600	830	710	390	21	37	43	55	61	59	..	890	2,020
10	91	170	380	250	440	670	...	11	34	27	53	54	44	..	780	610
11	110	200	240	320	400	500	180	18	23	33	41	54	47	30	510	1,240
12	92	300	480	630	780	800	670	20	36	42	66	57	60	67	1,650	3,940
13	110	200	130	130	420	360	630	32	..	21	27	..	56	70	1,350	1,940
31	96	160	190	140	160	260	...	..	..	..	..	..	..	..	550	670
32	120	190	180	270	210	190	350	..	..	..	..	..	..	..	740	1,270
33	100	270	320	480	470	490	690	..	..	..	..	..	..	..	1,540	1,860
34	85	210	200	270	220	260	280	..	..	..	..	..	..	..	490	990
35	110	190	380	320	290	450	620	..	..	..	..	..	..	..	570	3,640
36	120	280	260	490	460	430	660	..	..	..	..	..	..	..	650	1,820
37	100	170	230	150	260	600	710	..	..	..	..	..	..	..	610	1,510
38	100	280	240	340	330	520	440	..	..	..	..	..	..	..	880	1,750
77	89	310	380	770	990	1,040	1,040	23	32	54	63	64	60	42	3,130	2,710
78	78	96	210	360	570	500	560	14	54	60	54	77	53	28	1,030	930
79	71	420	190	650	770	850	600	20	55	..	51	62	51	60	1,720	1,000
80	59	130	200	230	290	430	340	10	35	66	57	56	49	45	1,470	510
81	79	830	490	630	940	1,070	960	11	51	71	81	64	55	40	2,500	9,090
82	67	310	74	320	690	690	580	16	43	48	49	46	52	34	1,830	630
83	130	300	490	460	630	1,070	1,180	14	56	45	63	60	59	42	1,900	4,080
84	74	360	400	660	750	750	750	22	49	41	54	56	60	39	2,000	6,630
85	73	100	150	240	380	640	540	21	20	43	47	..	52	38	1,440	1,000
Average	93	300	330	440	580	650	610	19	42	48	55	58	54	46	1,400	2,520

17. Member, S.; Bruger, M., and Oppenheim, E.: Arch. Path. **38**:210, 1944.

METHODS AND MATERIAL

Fifty female rabbits (Wistar strain) aged 3 to 5 months were employed. Twenty-four animals served as controls: 1 Gm. of cholesterol was added to the basal diet[18] three times a week for twelve weeks. In the experimental group, 1.5 to 2.5 mg. of desoxycorticosterone acetate[19] was administered intramuscularly to 26 rabbits three times a week on days when cholesterol was fed. The total cholesterol and the cholesterol esters of the whole blood were determined[20] every two weeks by Bloor's method as modified by Sackett.[21] At the end of the experimental period, the animals were killed, and the aorta and the liver were analyzed for cholesterol by the procedures previously described from this laboratory.[22]

RESULTS

The detailed findings appear in tables 1 and 2. It is apparent that parenteral administration of desoxycorticosterone acetate had little or no effect on the rise of blood cholesterol levels during the first eight weeks. Toward the end of the experimental period there was a slight inhibition of the elevation of blood cholesterol. The ester percentage of total cholesterol was not significantly different in the two groups. Table 3 represents a statistical analysis of the effect of desoxy-

TABLE 3.—*Statistical Analysis of Results*

Group	Specimens	Liver Cholesterol			Aorta Cholesterol		
		Mean	σ *	t †	Mean	σ *	t †
Cholesterol	24	1,510	± 560	....	3,050	± 2,150	....
Cholesterol + desoxycorticosterone acetate	26	1,400	± 790	0.46	2,520	± 2,270	0.80

* Standard deviation $\sigma = \sqrt{\frac{\Sigma (d^2)}{N}}$ where $\Sigma (d^2)$ represents the summation of the squares of the individual deviations from the mean and N the number of determinations.

† $t = \frac{\overline{X_1} - \overline{X_2}}{\sqrt{\frac{N_1 \sigma_1^2 + N_2 \sigma_2^2}{N_1 + N_2 - 2}}} \cdot \sqrt{\frac{N_1 N_2}{N_1 + N_2}}$ for a small series and t was checked against Fisher's tables. In this formula $\overline{X_1}$ represents the mean for the cholesterol-fed animals, $\overline{X_2}$ the mean for the cholesterol + desoxycorticosterone acetate group, N_1 the number of specimens in the cholesterol group, N_2 the number of specimens in the cholesterol + desoxycorticosterone acetate series, σ_1 the standard deviation of the cholesterol-fed animals and σ_2 the standard deviation of the cholesterol + desoxycorticosterone acetate group. In both the liver and the aorta cholesterol series the t values do not indicate a significant difference.

corticosterone acetate on the deposition of cholesterol in the liver and the aorta. In the control group the average amount of cholesterol in the liver was 1,510 mg. per hundred grams and the average amount in the aorta was 3,050 mg., while in

18. The basal diet was Ralston Purina® rabbit chow, which contains a mixture of grains and alfalfa hay supplemented with vitamins and minerals.

19. The desoxycorticosterone used was that marketed as percorten (Ciba)® 0.3 to 0.5 cc.

20. These determinations were made with the technical assistance of Samuel Member, B.S.

21. Sackett, G. E.: J. Biol. Chem. **64**:203, 1925.

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the treated group the averages were 1,400 mg. and 2,520 mg. per hundred grams, respectively. However, neither of these small differences for the small number of animals were statistically significant.

COMMENT

This study has demonstrated that desoxycorticosterone acetate has little if any inhibitory influence on the development of hypercholesteremia or on the deposition of cholesterol in the liver or the aorta of the normal young female rabbit fed cholesterol. In view of the conflicting results reported by previous investigators and in view of the fact that desoxycorticosterone acetate is only 1 of 28 compounds thus far isolated from the adrenal cortex [23] it is not permissible to interpret the negative results of this experiment as indicating that the adrenal cortex has no part to play in cholesterol metabolism. Particularly is this true since desoxycorticosterone acetate is primarily involved in mineral and water metabolism,[24] while the other steroids of the adrenal cortex affect the metabolism of organic substances.

CONCLUSIONS

Parenteral administration of desoxycorticosterone acetate has little or no effect on whole blood cholesterol levels in rabbits fed cholesterol.

Desoxycorticosterone acetate does not prevent the development of atherosclerosis of the aorta or the deposition of cholesterol in the liver of the rabbit under the conditions of the experiment.

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SILVERING OF LEPRA BACILLI IN TISSUES

F. LEÓN BLANCO, M.D.
HABANA, CUBA
AND
G. L. FITE, M.D.
CARVILLE, LA.

ALTHOUGH silver impregnations of tissues have been used previously [1] for the study of nerve changes in leprosy, and for the demonstration of reticulum, apparently silvering of the bacilli has not been observed. With the methods used by us, lepra bacilli have been found to be demonstrated routinely and effectively in biopsy material in more than 100 cases of leprosy.

PROCEDURES

The methods used have been those of Jahnel,[2] Levaditi,[3] and Manouélian,[4] modified in the manner to be described. Jahnels' method is longer and more tedious, but it has been found particularly effective, giving beautiful results in tissues fixed in formaldehyde or Bouin's fluid for as long as one year. The modification employed is, briefly, as follows:

1. Fix tissue in 4 per cent formaldehyde solution or Bouin's fluid for two weeks or more.
2. Place in pyridine for one to three days.
3. Wash in several changes of distilled water for twenty-four hours.
4. Fix in 4 per cent formaldehyde for four days.
5. Wash in several changes of distilled water for twenty-four hours.
6. Place in 95 per cent alcohol, several changes, for three to eight days.
7. Wash in distilled water until pieces of tissue sink to the bottom of the container.
8. Treat with aqueous silver nitrate (0.5 per cent in distilled water) in the dark at 37 C. for five to eight days. A copious amount of the solution should be used.
9. Wash in distilled water ten minutes to remove excess silver.

From the Patronato para la profilaxis de la lepra y sifilis, Habana, Cuba, and the United States Marine Hospital, Carville, La.

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2. Jahnel, F.: München. med. Wchnschr. **67**:932, 1920.

3. Mallory, F. B.: Pathological Technique, Philadelphia, W. B. Saunders Company, 1938, p. 193.

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10. Reduce for two days in a solution prepared as follows: pyrogallic acid, 4 Gm.; 37 per cent formaldehyde solution, 5 cc.; distilled water, 95 cc.

11. Dehydrate, clear, embed, section and mount without further staining, by any routine method.

RESULTS

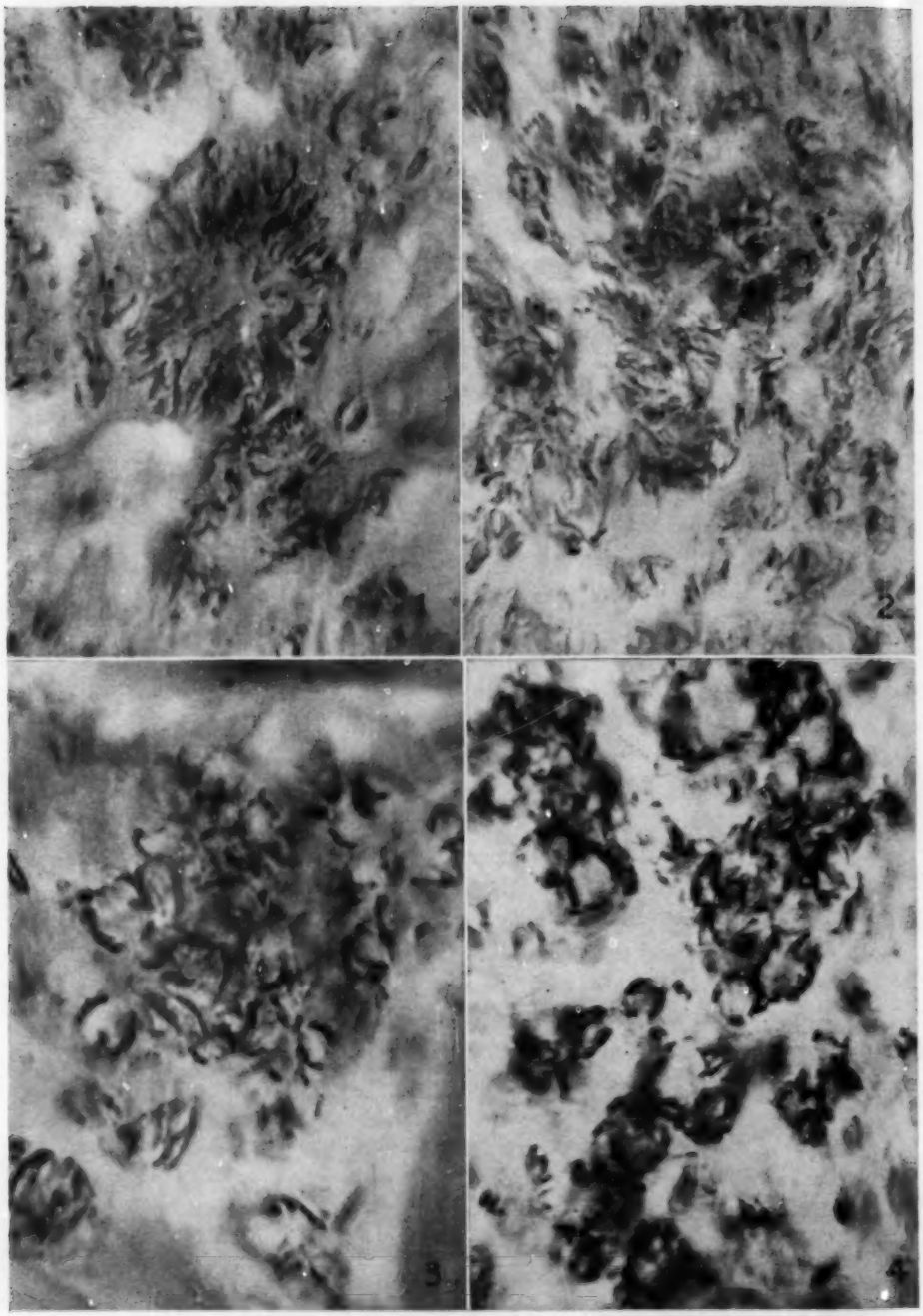
Lepra bacilli appear in various shades of black and brown, the majority in an intense black or blue-black. Differences of intensity are discussed later. Other structures, such as melanin and keratohyaline granules, are impregnated but are not confusing except close to the epidermis.

Because this method involves prolonged treatment of the whole tissue, the bacilli are extremely well fixed and are little or not at all disturbed by subsequent embedding and sectioning. The results, it is believed, produce a wholly true picture of the distribution and appearance of the bacilli in the tissues. Ordinary methods of revealing acid-fast bacilli and methods of silvering frozen sections produce much distortion, due to shrinkages, with bacilli frequently found in positions in which they could not reasonably occur. This distortion is absent from the embedded silvered preparations. Although the nuclei of the cells are not always well demonstrated, the relation of bacilli and cells is nonetheless well shown.

BACILLI IN THE MOST HIGHLY ACTIVE LESIONS

Such lesions are recognized histologically both from the large numbers of organisms present and from the character of the cells containing the organisms. These are not the vacuolated cells of the older leproma but are fairly simple macrophages of a wide variety of shapes and forms. In ordinary sections the parts of the cell occupied by the bacilli can be identified only by hollow areas in the cytoplasm which are not distinct vacuoles. Indeed, the numbers of organisms present would not be suspected, with the abundant and well stained cytoplasm.

In these lesions bacilli are seen in characteristic form (see figures 1 to 4). They occur in streams, or parallel columns, and in larger cells they radiate from the central part of the cell. They look not at all like the usually described "cigar packet" or "bundle" but resemble a small colony of a fungus with hyphae streaming out from a central point. Bacilli are not matted together but are separated by a well defined uniform narrow space. It becomes clear that the appearance of these fresh active lesions when stained with fuchsin methods is highly artefactual. In the latter case organisms are matted together and distorted in various ways. Hansen's original description (1874) of bacilli "crossing each other at very sharp angles" would not apply in this acute phase. This type of bacillary arrangement is limited to those lesions



Figs. 1 to 4.—Development of the vacuolated cells. Figures 1 and 2 illustrate the disposition of lepra bacilli in fresh active lesions. In figure 1 the bacilli radiate from the central part of the cell, which is occupied by the nucleus. Figure 3, from the same section as figure 1 shows an older area, with beginning vacuolation and bacilli curved about the vacuoles. Figure 4 shows a still older lesion with most of the bacilli ranged about vacuoles, or spread apart by the developing vacuoles, and moderate pleomorphism of the bacilli.

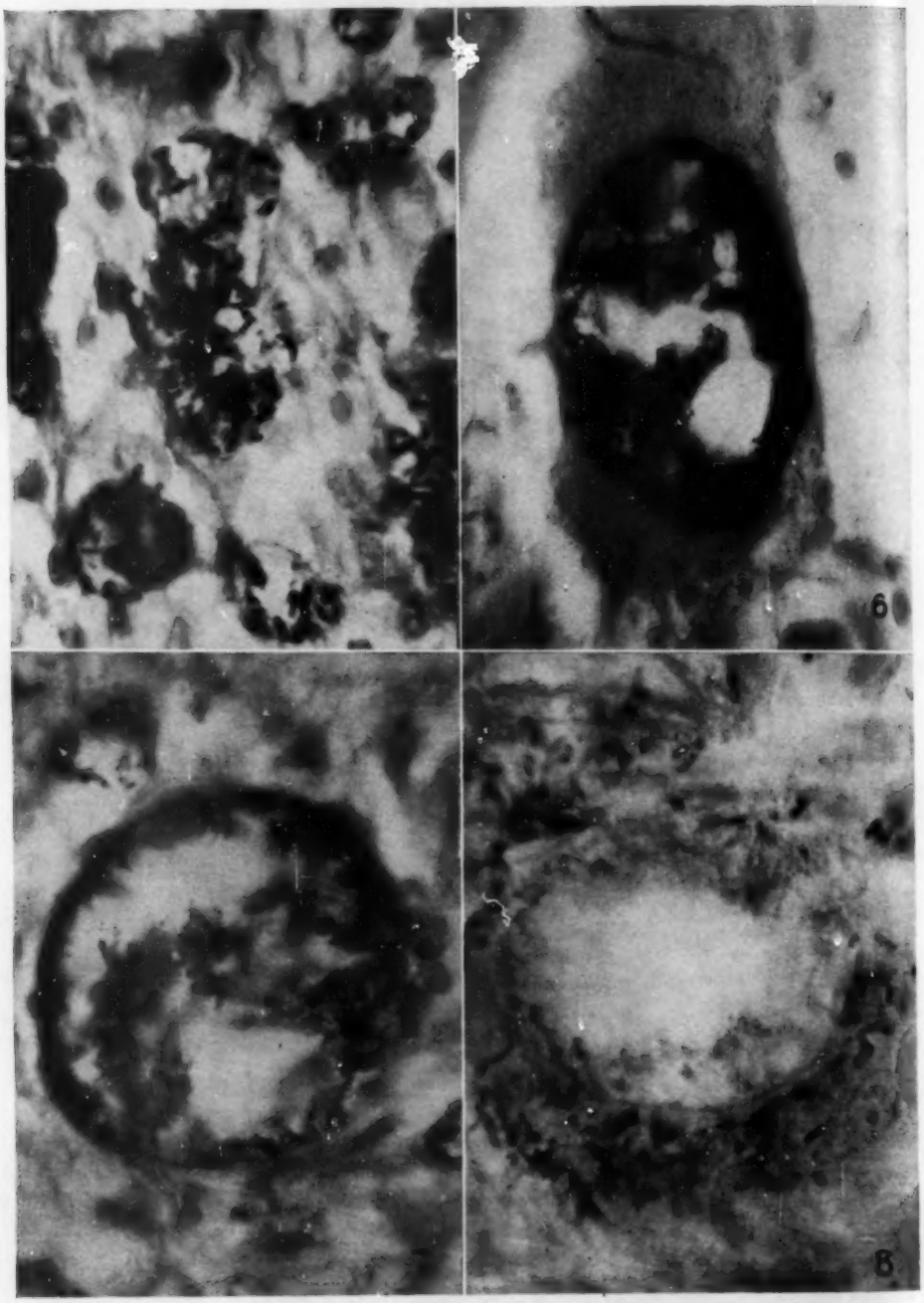
in which both bacilli and cells have been increasing in numbers, for which reason it is of especial importance. These are the growing, young bacilli.

In these lesions the bacilli appear quite long and nearly straight. They are uniformly, but not too deeply, silvered. They show little or no beading, although occasionally there is some greater condensation of the silver at the extremities of the organisms. The manner of the division of the organisms cannot be truly determined from a study of sections, but the impression is gained that in this case bacillary cell division must take place by transverse fission.

THE CHRONIC LEPROUS GRANULOMA

All stages between the highly and chronically active lesions are recognized. Of all lepromas that come under the microscope, the majority are of the chronic type, in which various degrees of vacuolation of the cells occur. There exists in leprology a popular misconception of the nature of these vacuoles, it being assumed frequently that they are the spaces occupied by the bacilli. This is not the case, as the silver impregnations show plainly. The vacuolated cells display a wide variation in size, the smallest with a single centrally placed nucleus, the larger with several nuclei anywhere in the cell. They are enmeshed in a reticulated, vascularized connective tissue framework, and in the silvered preparations the individual cell margin is demonstrated to a degree rarely observed in ordinary preparations. The bacilli are arranged in haphazard fashion in the cells. They lie between the vacuoles in what little cytoplasm is left in the cell, and the vacuoles are plainly seen as hollows between the meshwork of bacilli. The nucleus of the cell, which is commonly unsilvered by Jahnel's method but shown by Levaditi's, appears as a round space, with the bacilli lying closest to it markedly curved, their concave borders facing it. Bacilli in these cells show curved, bent, angulated forms of varying length, with variation in thickness of the individual bacillary body. Bacilli lying adjacent to vacuoles are also commonly curved about them.

It appears most likely that differences of appearance seen in bacilli in the chronic lepromatous lesion are partly determined by the development of the macrophage into the vacuolated cell with, at the same time, a marked diminution of the rate of growth of the bacilli. The organizing of the cells into a tissue is a factor in the shaping of the cells, which in turn affects the intracellular arrangements of the bacilli. The appearances of the chronic lesion suggest, even as do the clinical characters of leprosy, that in many cases the activity of the lesion is essentially stationary over long periods. The silvered preparations suggest that this is exactly the case, with only a slight degree of reproduction taking place in the highly vacuolated cell. The conception of the vacuolated



Figs. 5 to 8.—Development of the globus. Figure 5 shows highly vacuolated cells (well filled with bacilli) sharply set apart by the organization of the lesion. There is some coalescence of vacuoles. Such cells are forerunners of globi but do not necessarily develop into them. Figure 6 shows a well developed but young globus encased by a giant cell, bacilli well silvered throughout and moderate coalescence of vacuoles. Figure 7 shows a still older globus with weak silvering of bacilli, and figure 8 shows an old globus with complete coalescence of vacuoles and faintly silvered bacilli. Note, however, that there are well silvered bacilli in the encapsulating giant cell.

cell as a characteristic cell of leprosy is valid, but it must be recognized as a later result of intracellular growth. Vacuoles develop as the reproductive activity of the bacilli diminishes.

THE GLOBUS

The globus is a mass of bacilli, round or oval and sharply outlined. When large, it is often surrounded by a thinned-out giant cell, which may be so thin as to appear as a ring about the globus. The exact nature and significance of these structures and their manner of development have been much disputed, and the term is too frequently used to signify any large accumulation of bacilli in leprous tissues. From silvered preparations it is seen that the globus is indeed a characteristic structure following a definite evolutionary course.

In the fresh active lesions the structures commonly described under the heading of globi seem not to merit the term. There is no suggestion that the streams of actively growing organisms are developing into globi. In such cells the ordinary course is that in which vacuoles develop among groups of bacilli. The term "seed globi" applied to these groups of organisms by Cowdry [5] does not appear justified.

From the silver preparations, the globus appears to arise within a cell, or a group of cells fused together, after the cell has undergone degenerative changes with loss of nucleus and cytoplasm. There is always, in the bacillus-laden cell, much lipid material, which persists in the globus, as do also the vacuoles, so that the early or young globus presents much the appearance of the parent cell. Larger or older globi show fusion of the vacuoles, and the aged globus shows a single vacuole occupying most of the globus, with the bacilli distributed largely about the margin. In small young globi bacilli are routinely found well silvered. In old globi bacilli become progressively more weakly silvered. Bacilli near the rim of the globus frequently are better silvered than those more centrally placed. Sections of leprous tissues containing globi stained by fuchsin show, on the other hand, the bacilli to be equally stained throughout the globus.

The globus frequently becomes something of a foreign body. In a definite small percentage of cases of leprosy there is formation of giant cells throughout the lesions. Many of these are of the foreign body type, and they routinely enclose globi. In regressive leprous lesions the globus is seen to be a highly persistent structure, usually with some form of multinuclear cell response to it. From the silvered sections it is seen that many bacilli in globi lose the affinity for silver, suggesting an alteration of their chemical structure. This is interpreted as a degenerative change, although the bacillus is preserved morphologically.

5. Cowdry, E. V.: Am. J. Path. **16**:1940, 103.

Globi are by no means uniformly observed in leprous lesions, and circumstances leading to their formation appear to be somewhat accidental therefore. The extremely large globi rarely observed seem to arise from coalescence of many cells, not from enlargement of the formed globus. The density of organisms in the globus naturally corresponds with the density of bacilli in the cells from which the globus arose.

DEGENERATIVE FORMS OF BACILLI

Weakly silvered and nonsilvered bacilli are readily identified in globi. Similar bacilli are also commonly encountered in minor numbers in almost every chronic lesion. They are seen in areas in which well silvered bacilli also occur, with all degrees of silvering observable. Both silvered and nonsilvered bacillary fragments are also seen. It seems clear that these are degenerate forms of the organisms. They may be fairly numerous in small areas, and their presence supports the idea that in the chronic leproma death and degeneration of bacilli are constantly taking place in some areas, even though other areas of the same lesion show evidences of progression. This is readily observed in large solid lepromas, in which the deeper areas show regression, while the most superficial ones show progression. It is suggested that well silvered organisms are healthy organisms; poorly silvered ones, degenerate or dead organisms. Obviously, such a conclusion can be drawn only on a broad basis from those specimens in which many degrees of silvering are observed in the same section. Unless absolute uniformity of technic is maintained, there will be differences in the degree of silvering in different blocks.

Pleomorphism of the lepra bacillus is marked in the chronic lesions but barely noticeable in the fresh lesions. It is especially prominent in areas in which degenerate forms occur, and is regarded as being related to the regressive phase of leprosy, not to the growth phase of the organisms. Study of such organisms has, in the past, led to erroneous conclusions with regard to the essential morphologic aspects of the lepra bacillus.

HALOS SURROUNDING BACILLI

In silvered preparations a halo is routinely seen about the bacillus as a narrow clear space investing the organism. It appears not to be a phenomenon of spherical aberration. However, halos are also encountered in fuchsin-stained sections and may be observed in sections of lesions containing bacteria quite unrelated to leprosy, so that their significance seems small. It is important to stress that the halos do not fuse with the vacuoles in the cells and that they are not to be regarded as such.

SUMMARY AND CONCLUSIONS

Lepra bacilli are readily silvered in blocks of lepromatous tissues with Jahnel's and other methods.

Bacilli silvered in the block undergo a minimum of distortion and yield a true picture of the infection of the tissues, scarcely obtainable with other methods. While such silvering is not practical as a routine clinical-pathologic procedure, it is highly valuable in the study of the lepromatous lesion.

Study of silvered sections shows that past concepts of the morphologic aspects and arrangements of bacilli are open to considerable revision. Actively growing organisms resemble minute colonies of fungi, and do not appear as "bundles" or "cigar packs." A new concept of the nature of the globus is offered.

Different degrees of silvering observed indicate that the weakly silvered bacillus constitutes one form of the degenerating organism.

SPLENIC CYSTS

H. T. TAMAKI, M.D.
NORRISTOWN, PA.

A PERUSAL of the medical literature on splenic cysts discloses that they are rather uncommon. The fact that they are rare can be attested to by the fact that not a single case of splenic cyst has been recorded in the surgical file at Jefferson Medical College Hospital since 1915. During a thirty year period from 1904 to 1934, Pemberton reported, only 4 cases of cysts of the spleen have been seen at the Mayo Clinic [1] in approximately 800 splenectomies. Denneen [2] reported that at the Bellevue Hospital in New York they were able to find record of but 8 cases of splenic cysts, none of which were of the hemorrhagic type, and that no cases were recorded at St. Vincent's Hospital during the thirty year period prior to 1940.

Reports of cases of cystic disease of the spleen in the American literature have been extensively reviewed by Fowler.[3] According to Fowler, the first case of cystic tumor of the spleen was reported by Andral in 1829. Fowler,[3b] in 1912, collected 89 cases of nonparasitic cysts of the spleen. In recent years Frank [4] has written extensively on this subject and brought the total number of cases reported up to 1930 to 111. Other recent reviews on the subject have been written by Benton,[5] Sherwin [6] and Shawan.[7]

The total number of nonparasitic cysts reported up to 1939 was 137, according to Fowler's most recent review.[3a] By 1942 McClure and Altemeier [8] raised the total to 149. Gallagher and Mossberger [9] described the one hundred and fifty-fifth nonparasitic cyst, and by the end of 1944 Harmer and Chalmers [10] had increased the reported cases

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From the Department of Pathology, Montgomery Hospital.

1. Roberson, F.: Ann. Surg. **111**:848, 1940.
2. Denneen, E. V.: Ann. Surg. **116**:103, 1942.
3. Fowler, R. H.: (*a*) Internat. Abstr. Surg. **70**:213, 1940; (*b*) Ann. Surg. **57**:658, 1913; (*c*) **74**:20, 1921; (*d*) **80**:58, 1924.
4. Frank, L. W.: South. M. J. **23**:212, 1930.
5. Benton, R. W.: J. A. M. A. **99**:1674, 1932.
6. Sherwin, B.; Brown, C. R., and Liber, A. F.: Ann. Surg. **109**:615, 1939.
7. Shawan, H. K.: Arch. Surg. **27**:63, 1933.
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9. Gallagher, P., and Mossberger, J. I.: Ann. Surg. **116**:933, 1942.
10. Harmer, M., and Chalmers, J. A.: Brit. M. J. **1**:521, 1946.

to 163. Since then at least 4 other cases have been reported [11] in the American literature, which would make the case I report the one hundred and sixty-eighth.

REPORT OF CASE

E. B., a 15 year old white boy, a student, was admitted to Montgomery Hospital on Oct. 6, 1948. He was referred to the hospital by the school physician, who noticed a large abdominal mass in the left upper quadrant on routine physical examination. The patient complained of nothing other than an occasional slight "pulling sensation" over the area of the mass. He first noted a slight enlargement just beneath the margin of the left rib approximately four years before, following an accident in which he fell from a tree, injuring his abdomen and sustaining a fracture of the left wrist. At that time the possibility of a ruptured spleen was entertained, and the surgeon introduced a needle into the peritoneal cavity, but obtained no blood. The mass remained small until three months prior to admission, when there was a gradual and fairly rapid increase in its size.

Examination disclosed a well developed, well nourished white youngster in no apparent distress. The head and neck appeared normal. A large mass was palpable in the left upper quadrant of the abdomen, which had produced a flaring of the lower ribs on the left side. The mass was easily visible and extended approximately 14 cm. below the costal margin. A peculiar resilience was noted on palpation. No definite fluid wave could be elicited, and the mass moved slightly with respiration. The rest of the examination disclosed nothing remarkable. Temperature, pulse and respiration were normal.

Urinalysis revealed a trace of albumin and occasional leukocytes. The hemoglobin content was 88 per cent; the red blood cell count was 4,530,000; the white blood cell count, 6,300, with 71 per cent polymorphonuclear leukocytes, 28 per cent lymphocytes and 1 per cent eosinophilic granulocytes.

The usual roentgen survey films of the chest and abdomen showed the lung fields clear, the cardiac shadow normal in size and configuration, and a definite elevation and splinting of the left leaf of the diaphragm. The roentgenogram of the abdomen showed a spherical mass in the left upper quadrant, with a rather sharply defined inferior border, which extended to the level of the body of the fourth lumbar vertebra. There was a curvilinear shadow of a calcified region extending transversely across the mass some 2 inches (5 cm.) above its inferior border. The stomach contained sufficient gas to demonstrate that it was displaced toward the right side, while the splenic flexure of the colon was displaced downward. The right kidney was normal in size, shape and position, while the left was rotated on its long axis and displaced downward, with both poles lying in the same horizontal plane. These findings were corroborated by an intravenous pyelogram and a roentgen study of a barium sulfate enema (Dr. R. D. Campbell).

Operative Note (Dr. D. M. Headings).—A long subcostal incision was made, the left rectus muscle was cut transversely and the peritoneum was opened. A large splenic cyst was discovered, which was mobilized and posterior fibrous adhesions were severed. The entire mass was enucleated in an intact state. The patient left the operating room in good condition. He had an uneventful course postoperatively and was discharged on the twelfth day.

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11. (*a*) Jameson, E. M., and Smith, O. F.: U. S. Nav. M. Bull. **45**:537, 1945. (*b*) Neidhardt, H. W.: J. Kansas M. Soc. **45**:382, 1944. (*c*) Duggan, H. E.: J. Canad. M. Serv. **2**:283, 1945. (*d*) Duby, H.: New England J. Med. **237**:731, 1947.

Pathologic Report.—The specimen consisted of a large ovoid cyst, measuring 20 by 23 by 14 cm. and weighing 3,300 Gm. It was received intact. It presented in the main a dark purplish red surface, owing to the thinned-out condition of the normal splenic tissue over the cyst. The portion of the cyst which was posterior presented a thin, grayish red wall with no surrounding splenic tissue. This area also harbored numerous fibrous adhesions. At both poles there was a margin of grossly normal spleen. At one end it presented a narrow shelf 1.5 cm. in thickness and stretching out for a distance of approximately 8 cm. At the other pole there was a flat pyramid-shaped portion of the spleen, measuring 15 by 5 by 3 cm. The lumen of the cyst was filled with approximately 2.5 liters of dark brown, opalescent fluid. Examination of this fluid disclosed numerous red cells and many cholesterol crystals and necrotic debris. After the fluid content had been emptied out, numerous soft, light to dark gray nodules, varying in size from 1 to 5 cm. in longest dimension, were seen loose within the lumen (fig. 2). They were extremely soft and were apparently necrotic coagulum. The cyst wall was translucent where it was not covered by splenic tissue. In the main, however, it was surrounded by a layer of splenic tissue. The inner aspect of the cyst presented a light gray surface with a considerable degree of trabeculation. There was necrotic fibrinous material adherent to portions of the wall.

Section of the cyst wall disclosed a thick laminated hyalinized fibrous tissue. It was lined in part with a single layer of endothelium-like cells. There were areas, however, where the lining epithelium was not discernible. Within the wall were a few scattered foci of calcification. On the external aspect of the cyst wall there was a layer of compressed splenic tissue. There was moderate distortion of the normal splenic structure, also marked diminution of the number and size of the malpighian corpuscles. The surrounding reticulum, fibers and pulp were compressed and in many areas replaced by fibrous tissue with some degree of hyalinization. The infiltrate was made up primarily of lymphocytes along with numerous polymorphonuclear leukocytes. Eosinophilic granulocytes were quite prominent. There were a few foci of hemorrhage within the stroma. The splenic capsule disclosed a slight degree of hyaline change.

The sections taken through the necrotic material within the lumen disclosed a homogeneous light pinkish-staining material harboring countless cholesterol crystals with a sprinkling of polymorphonuclear leukocytes.

Diagnosis.—Hemorrhagic cyst of the spleen.

INCIDENCE

Various classifications of splenic cysts have been suggested in the literature. Such cysts have been classified according to morphologic aspects, pathogenesis, cell lining and contents; but each classification has its faults and limitations. The simplest classification would be to divide all splenic cysts into parasitic and nonparasitic groups. According to Fowler,[3a] echinococcus cyst occurs about twice as often as nonparasitic cysts. The nonparasitic cysts may be further subdivided into primary and secondary cysts. Of the nonparasitic cysts, secondary cysts are approximately four times as common as primary cysts, as evidenced by Fowler's study of a large series of cases in which he found that 21 per cent were true cysts and 79 per cent false cysts.

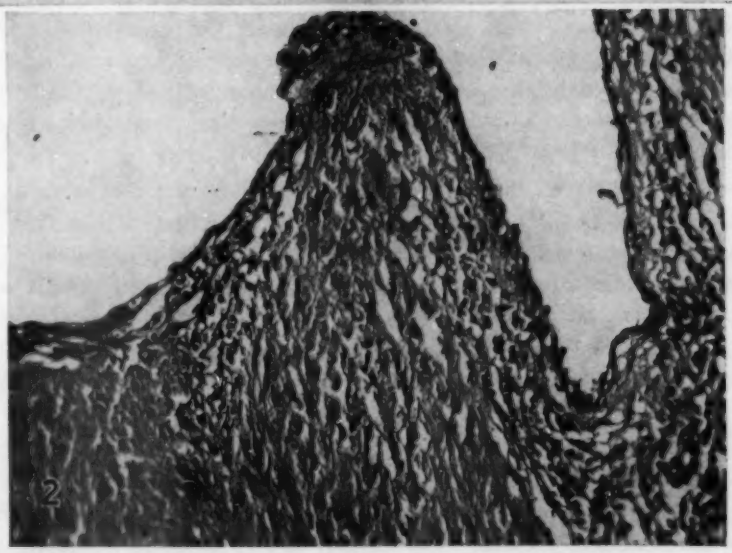

Fig. 1.—Large cyst of the spleen of a 15 year old boy, containing nodules (apparently necrotic coagulum).

Fig. 2.—Photomicrograph of the wall showing endothelium-like lining.

It is the purpose to confine the discussion primarily to the secondary or false cysts, which are of the greatest clinical importance. These secondary or false cysts may be either single or multiple. In nearly 80 per cent of the cases of nonparasitic cyst a single cyst is found. It may be subcapsular or situated deeply within the organ, the former type being nearly twice as common. Such cysts vary considerably in size; one may measure as little as a few centimeters in diameter, and another may fill the greater part of the abdominal cavity. Hamilton and Boyer [12] reported a hemorrhagic splenic cyst which contained 7 liters of fluid, and Heurtaux [13] described one containing 10 liters of fluid. Few of the reported solitary nonparasitic cysts were calcified. Snoke,[14] in 1943, was able to find but 7 cases of solitary calcified cysts of the spleen described in the literature. Since then Neidhardt [11b] and Duggan [11c] have reported 2 more cases, making the present case the tenth case of calcified solitary false cyst. Secondary cysts of the spleen may be found at any age. The majority fall between the ages of 20 and 40, and 65 per cent are said to occur in females.

CAUSES

In the consideration of the etiologic factors of splenic cysts it is agreed by all that trauma is important. In the reviews of others, as well as in a review of the more recently reported cases, a history of trauma can be obtained in nearly one fourth of the cases. The interval between the injury and the development of the cyst was variable, although in fully 50 per cent of the cases the cyst occurred within three years and in nearly 90 per cent within ten years.[3a] The trauma was usually a fall on the abdomen, as in our case, or a direct blow over the splenic area.

Of the cysts with history of trauma, nearly 80 per cent were of the hemorrhagic type. McClure and Altemeier [8] suggested that they are actually encysted hematomas, which result from an injury which fails to produce laceration of the surface of the spleen but which tears deep-seated vessels and causes hemorrhage. In time a cyst is formed with a bloody fluid content surrounded with a fibrous wall. It is also possible for intrasplenic hemorrhage to occur spontaneously, without any antecedent history of trauma.

Menstruation and pregnancy appear to play some part in the formation of splenic cysts. Of the cysts occurring in women, the majority were found during the child-bearing age. They have been described in women with menstrual irregularities as well as in pregnant women. According

12. Hamilton, C. S., and Boyer, E. H.: Ann. Surg. **73**:58, 1921.

13. Heurtaux, A.: Bull. et mém. Soc. de chir. de Paris **24**:928, 1898.

14. Snoke, P. O.: Am. J. M. Sc. **206**:726, 1943.

to Mannier (quoted by Frank[4]), the instance in which such a cyst occurs during the reproductive period is explained by the fact that the spleen becomes congested in pregnancy and during menstruation. In support of this theory, Frank[4] described a case in which a large cyst was marsupialized, and blood was discharged from it at each menstrual period after the operation. This, however, is not convincing proof of a direct relationship, as there is some doubt as to the origin and the nature of the cyst described. There are other reports of cases in the literature to suggest that the cyst of the spleen is related to menstruation, such as those in which metrorrhagia was relieved after splenectomy.[15] The unconvincing evidence given would lead one to question a direct relationship of menstruation or pregnancy and formation of a splenic cyst. As Fowler suggested, congestion during menstruation may result in an increase in size of a preexisting cyst. Infarctions of the spleen may possibly be related to pregnancy with resulting secondary hemorrhage and cyst formation. Frank[4] expressed the belief that splenic cysts are all serous in type in the beginning but that some become blood cysts because of the bleeding into the cyst which may occur as the result of splenic congestion associated with menstruation.

The possibility that the splenic cyst is related to some antecedent disease has been discussed by some writers. Malaria is mentioned most frequently as a predisposing cause. According to Harmer and Chalmers,[10] splenic cyst is not uncommon in West African natives, in whom malaria is almost universal. Other less frequently associated diseases are syphilis, typhoid, paratyphoid and mumps. In all probability the relationship is perhaps indirect, in that the splenomegaly associated with these diseases would more readily subject the organ to trauma.

Embolism or thrombosis of the splenic vessels has been shown to cause cyst formation, and this type has been described recently by Dabrzaniecki.[15] Cysts may also occur within the splenic parenchyma as a result of endarteritis.

Snyder and Rezek[16] favored the theory of malformation, basing their opinion on the fact that congenital malformations may become manifest later in life through postembryonic influences.

PATHOLOGIC ASPECTS AND GENESIS

Large splenic cysts may be lymphogenous or hemorrhagic in origin. The contents, therefore, may be serous, serosanguineous or purely hemorrhagic in nature. The blood may be coagulated, black to brown and frequently possessed of a sparkling sheen due to the presence of

15. Dabrzaniecki, W.: Ann. Surg. **92**:67, 1930.
16. Snyder, J. W., and Rezek, P. R.: South. M. J. **36**:263, 1943.

cholesterol crystals. There is considerable variation in the thickness of the cyst wall. Some older cysts may lack a true wall. The inner surface may be smooth, rough or trabeculated. The wall itself is made up essentially of dense connective tissue, which may in some cases harbor foci of calcification. Approximately 9 per cent of the reported cysts were calcified.[2] Surrounding the cyst wall there may be a stretched-out layer of splenic tissue of varying thickness. Depending on the degree of intracystic pressure, the structure of the splenic tissue may or may not be distorted. In the area of great pressure there may be signs of atrophy with considerable compression of the reticulum fibers and pulp and decrease in size and number of the lymph follicles.

Microscopic study of the solitary hemorrhagic cysts discloses a laminated dense hyalinized connective tissue wall which may or may not present a cellular lining. Although secondary or false cysts are said not to possess a true epithelial lining, a single layer of endothelial cells or even a stratified squamous epithelium has been described in numerous reports.[17] The nature of the lining cells depends on different factors, such as cause, size and age of the cyst. It is conceivable that serous cysts may arise from lymphatic vessels; or, if the presence of lymphatic channels in the spleen be denied, the endothelial lining could be derived from the endothelial cells of the spleen or possibly from splenic inclusions of the investing peritoneal endothelium. Even in a primary blood cyst an endothelial lining may be acquired,[17a] as demonstrated in my case. In all probability the squamous cell lining described resulted from the metaplasia of endothelial cells.

DIAGNOSIS

In many instances the patient with a splenic cyst is asymptomatic, particularly if the cyst is small. Not infrequently, however, as was observed in my case, even a large cyst may cause minimal or no symptoms at all. Symptoms of pressure are the usual complaints with the large cysts. They may express themselves as a feeling of heaviness or a dragging sort of pain. There may be dyspepsia or inability to eat a large meal, owing to the compression of the stomach and intestines. More rarely, dyspnea, constipation or diarrhea may be present.

Although Sweet [18] stated that "large cysts present a clinical picture which is so characteristic that the diagnosis can be readily made," the diagnosis of splenic cyst was rarely made in the reported cases. In the present case the preoperative diagnosis of splenic cyst was made by some of the physicians who saw the patient in consultation.

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17. (*a*) Paul, M.: Brit. J. Surg. **30**:336, 1943. (*b*) Shawan,[7e] Jameson and Smith.[11a]

18. Sweet, R. H.: New England J. Med. **228**:705, 1943.

Perhaps the greatest help in diagnosis can be had from careful physical and roentgen examinations. Palpation of the abdomen may disclose a tumor in the left upper quadrant of the abdomen. If the cyst is large, the costal margin of the overlying left rib is usually pushed outward. This is not a usual finding with cyst of the pancreas or the ovary. At times the cystic nature of the tumor may be suspected from the doughy sensation. The roentgen examination may be the determing factor in many cases.[19] A flat roentgen plate of the abdomen may disclose that the left side of the diaphragm is high, and the motion may be seen to be impaired when observed by fluoroscope. Occupying the left upper quadrant may be a large soft tissue mass either with or without foci of calcification. In practically all reported cases of large cyst of the spleen the roentgenograms show the stomach to be pushed to the right and backward and the splenic flexure to be displaced downward and to the right. Occasionally there may be some displacement of the left kidney, usually downward. Although the renal pelvis is not usually altered, there may be some distortion due to the downward displacement. Obliteration of the psoas muscle on the left side is also described. It is interesting to note that within four weeks after splenectomy in cases of splenic cyst the displaced organs are back in their normal position.

DIFFERENTIAL DIAGNOSIS

In considering the differential diagnosis of cyst of the spleen, one must consider cyst of the kidney, the ovary, the pancreas, the mesentery or the omentum, as well as cyst of the left lobe of the liver. Other causes of splenomegaly must also be considered along with the rare retroperitoneal tumor.

Of some help in the diagnosis of splenic cyst is the observation that it is usually more superficial than one would ordinarily find a renal tumor to be. The pancreatic cyst, as well as the ovarian cyst, occupies a lower position in the abdomen. Sweet [18] stated that the outward flaring of the left costal margin does not occur as a rule in the case of pancreatic cyst and rarely in that of ovarian cyst. Ostro and Makover [20] described a case of chronic myelogenous leukemia with marked splenomegaly, in which the spleen extended into the pelvis and to the midline. As contrasted with their roentgenograms of splenic cyst, those made in a case of leukemia disclosed that the left side of the diaphragm was in normal position, with no spreading of the ribs, no displacement of the kidney and no obliteration of the psoas muscle. Furthermore, although the descending colon was displaced to the right, the splenic flexure, as well as the cardiac end of the stomach, was in normal position.

19. Elkeles, A., and James, J. I. P.: Brit. J. Radiol. **16**:59, 1943.

20. Ostro, M., and Makover, H. B.: Am. J. Roentgenol. **37**:782, 1937.

TREATMENT

There is but one method of choice for the treatment of splenic cysts. Except in those instances in which adhesions may make it inadvisable, splenectomy should be performed in all cases of large cyst of the spleen. Over an extensive series of cases a mortality rate of approximately 4 per cent was reported for cases in which splenectomy was done.[3a] Various other methods of treatment have been tried—marsupialization, puncture of the cyst, coagulation of the contents and extirpation of the cyst—but the results have been generally unsatisfactory.[5]

SUMMARY

A review of the literature discloses reports of 167 cases of splenic cyst. An additional case is reported. The etiologic and pathologic aspects, as well as the diagnosis and treatment, of cysts of the spleen are discussed.

EFFECTS OF FOLIC ACID DEFICIENCY AND A FOLIC ACID ANTAGONIST ON CHICKS

EPHRAIM WOLL, M.D.
BURLINGTON, VT.

THE isolation, the synthesis and the clinical application of folic acid and its derivatives opened new roads to the study of nutrition, cellular metabolism and certain aspects of tumors. General reviews of the subject have been made by Jukes and Stockstad [1] and Sargent.[2] Recent studies of folic acid deficiency have been reported by Cartwright and associates [3] and Rinehart and Greenberg.[4] The first clinical application of folic acid conjugates and antagonists in the treatment of cancer was made by Farber and co-workers.[5] The effects of folic acid, folic acid deficiency and folic acid antagonists on Rous sarcoma were described by Little and associates [6] and Woll.[7]

In the latter study pathologic changes were seen in the deficient and the antagonist-treated animals. The purpose of the experiments to be described was to expand these observations and to learn the sequence of events in terms of morphology.

MATERIALS AND METHODS

Approximately 200 1 day old New Hampshire Red chicks were used. The choice of very young birds was guided by a desire to have rapidly growing subjects in which nutritional disturbances may manifest themselves sharply. All

From the Research Department, Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y., and the Pathology Department, Children's Medical Center, Boston.

1. Jukes, T. H., and Stockstad, E. L. R.: Physiol. Rev. **28**:5, 1948.

2. Sargent, F., II: New England J. Med. **237**:667 and 703, 1947.

3. Cartwright, G. E.; Fay, J.; Tatting, B., and Wintrobe, M. M.: J. Lab. & Clin. Med. **33**:397, 1948.

4. Rinehart, J. F., and Greenberg, J. D.: Am. J. Path. **24**:710, 1948.

5. Farber, S.; Cutler, E. C.; Hawkins, J. W.; Harrison, J. H.; Peirce, E. S., II, and Lenz, G. G.: Science **106**:619, 1947. Farber, S.; Diamond, L. K.; Mercer, R. D.; Sylvester, R. F., Jr., and Wolff, J. A.: New England J. Med. **238**:787, 1948.

6. (*a*) Little, P. A.; Sampath, A.; Paganelli, V.; Locke, E., and Subbarow, Y.: Tr. New York Acad. Sc. **10**:68, 1948. (*b*) Little, P. A.; Sampath, A., and Subbarow, Y.: J. Lab. & Clin. Med. **33**:374, 1948.

7. Woll, E.: Tr. New York Acad. Sc. **10**:83, 1948.

the chicks received water, a diet and certain compounds to be described. They were divided equally into the following dietary groups:

Group 1 (control)—a commercial chick feed.

Group 2—a synthetic folic acid–free diet developed by Dr. J. J. Oleson, of our laboratories.

Its composition is as follows:

Cerelose (dextrose monohydrate)	53.0%	Nicotinamide	3 mg. %
Alcohol-extracted casein	22.0%	Pyridoxine	0.5 mg. %
Salt mixture	4.3%	Thiamine hydrochloride	0.3 mg. %
Calcium gluconate	3.0%	Biotin	0.03 mg. %
Gelatin	8.0%	Riboflavin	0.5 mg. %
Ruffex (purified cellulose containing 70% alpha cellulose)	4.0%	Inositol	100 mg. %
Soy-bean oil	5.0%	p-aminobenzoic acid	5 mg. %
Cholic acid	0.25%	Alpha tocopherol (vitamin E)	5 mg. %
Cystine	0.45%	Vitamin K	0.2 mg. %
Choline chloride	200 mg. %	Vitamin A	3,500 units %
Calcium pantothenate	3 mg. %	Vitamin D	200 units %

Group 3—the synthetic diet plus a daily intraperitoneal injection of 100 micrograms of folic acid in aqueous solution.

Group 4—a commercial chick feed plus a daily intraperitoneal injection of 0.1 mg. of 4-aminopteroylaspartic acid.

Group 5—a commercial feed with 80 mg. of 4-aminopteroylaspartic acid mixed in each kilogram of feed.

Group 6—the synthetic folic acid–free diet for ten days, followed by the same diet plus a daily intraperitoneal injection of 0.1 mg. of folic acid for the next five days.

The doses of folic acid and antagonist were judged optimal on the basis of the extensive experience of Little and co-workers [6a] in the study of nutritional control of the Rous sarcoma.

Three to 5 birds from each group were killed by decapitation on the second, third, fourth, fifth, seventh, tenth, twelfth, fifteenth, seventeenth and twentieth days of the experiment and examined immediately. Blocks of tissue were taken from all the viscera and from pectoral muscle, skin, tongue and tibial marrow. In a few cases blocks were removed also from the brain and the spinal cord. Numerous fixatives and stains were used. Fixation in formaldehyde–Zenker solution, paraffin embedding and hematoxylin-eosin staining were found to be a most useful procedure. In certain cases, iron, fat and connective tissue stains were of special value. Care was taken to obtain areas that would be anatomically comparable as to location and as to plane of section. This was particularly important in the study of the marrow, which was removed by snipping off the proximal epiphysis and then, by gentle pressure and traction, obtaining a fairly intact and uniform pencil of tissue. With the older birds, longitudinal splitting of the bone was required. No decalcification of the marrow block was necessary. Animals which died during the experiment were generally not examined, as it was considered that the histologic appearances would be unreliable.

In order to learn the effects of malnutrition in general, 20 chicks were given a commercial feed but in much reduced amounts. Thus the birds which weighed 35 to 40 Gm. on the first day of the experiment weighed only 30 Gm. at the end of ten days, compared with 55 to 60 Gm. for the controls. This group was killed and examined in the same way as the others.

An additional group of 10 birds received a total of 200 micrograms of 4-aminopteroylaspartic acid in two days by intraperitoneal injection. These were killed on the third day and treated similarly to the other groups.

GROSS OBSERVATIONS

Group 1 (control).—There were no abnormalities of development or of nutrition, nor was there evidence of spontaneous disease.

Group 2 (folic acid–free diet).—The chief findings were progressive emaciation and retarded development of the body and the viscera. The development of the body and the viscera was estimated to be 50 to 60 per cent of the normal on the fifteenth to twentieth days. There was no appreciable delay in the appearance of the wing feathers or the comb. The blood was watery and pale red. The marrow, which was at first pale red and more moist than normal, became light brown and viscid. The bowel, especially the distal third, including the ceca, was often but not always markedly distended with foul-smelling, abnormally watery feces. Grossly there was no appreciable increase in its fat content. The yolk sac was not any smaller than those in comparable controls. The gallbladder was sometimes distended with dark green, viscid bile.

Group 3 (folic acid–free diet plus folic acid by injection).—There were no grossly appreciable differences from the control birds.

Group 4 (regular diet with 4-aminopteroylaspartic acid in the diet).—The changes were similar in all respects to those obtained with the folic acid–free diet. However, the lesions were less pronounced and appeared later. From the fifteenth day on, several birds appeared to have recovered and to be almost normal except for a slightly smaller size of body and viscera.

Group 5 (regular diet plus 4-aminopteroylaspartic acid by injection).—The changes were the same as in the folic acid–free group. Toxic signs developed more rapidly and led to the death of approximately 10 to 15 per cent of the birds before the twentieth day.

Group 6 (folic acid–free diet for ten days, followed by same diet supplemented with a daily intraperitoneal injection of 0.1 mg. of folic acid).—The findings to the tenth day were identical with those of the similar group already described. From the twelfth day, i. e., after the feeding had been supplemented parenterally with folic acid, there was a rapid and marked improvement in nutrition and development. However, by the fifteenth day recovery was not complete.

The starved animals were malnourished, anemic and underdeveloped. The yolk sac was much smaller in these than in the controls, while the gallbladder was often distended. The tibial marrow was pale, slightly viscid or watery but not light brown.

The animals which received a large amount of the antagonist in two days appeared stuporous before death. At autopsy no notable gross abnormalities were seen.

MICROSCOPIC ORSERVATIONS

General Considerations.—There is a rapid change in the gross and the microscopic anatomy of the chick in the first weeks of life. For example: Granulopoiesis, at first slight and seen only along the endosteal and the epiphysial margins, becomes pronounced in the first three weeks and permeates the whole marrow (fig. 1). Foci of small mononuclear cells, considered by some investigators to be thrombocytes, appear in the second and third week. The amount of fat in the liver decreases steadily. Little or no fat is present in the usual depots. Thus, to evaluate properly the pathologic condition seen, it is important to compare the treated birds with the normal controls from day to day and, as far as possible, to compare planes that are anatomically equivalent even from a microscopic standpoint.

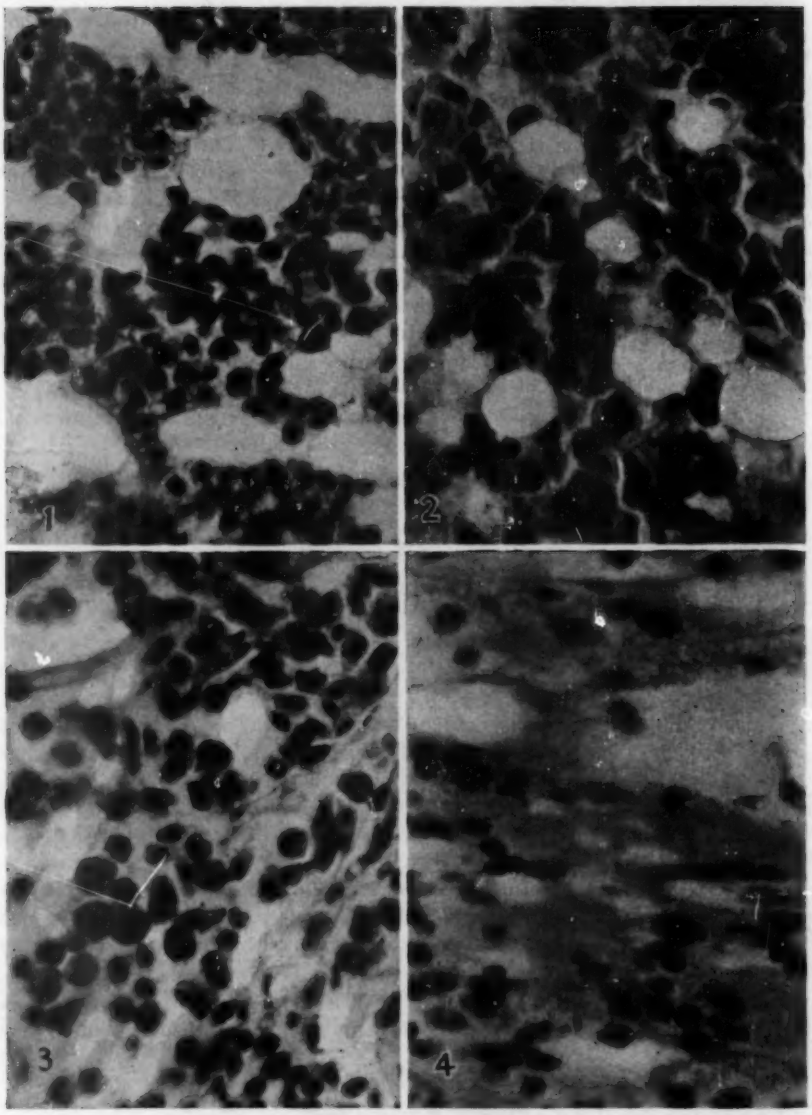

Fig. 1.—Marrow of a normal 15 day old chick. Note the proportion and the size of the various blood cell elements. The cells with deep-staining nuclei are erythrocytes. The white cells show cytoplasmic granules or rods. The blast cells have a homogeneous cytoplasm and a stippled nucleus. Hematoxylin and eosin; × 650.

Fig. 2.—Marrow of a 15 day old chick that was restricted to a folic acid–free diet. Note the definite decrease in number of erythrocytes and the increase in size and number of immature red and white blood cells. Hematoxylin and eosin; × 650.

Fig. 3.—Marrow of a 15 day old chick which was treated with 4-aminopteroyl-aspartic acid by parenteral injection. Note the severe pancytopenia. Hematoxylin and eosin; × 650.

Fig. 4.—Marrow of a 17 day old chick restricted to a folic acid–free diet. The aplasia and the myxomatous character of the stroma are seen. Hematoxylin and eosin; × 650.

The changes in the folic acid–deficient birds and those in the birds treated with the antagonist were found to be essentially the same. They will therefore be described together. The differences and similarities of these birds as compared with the malnourished group will be described later. The most impressive departures from normal were seen in the bone marrow and the bowel.

Bone Marrow.—As early as on the second day there was a sharp reduction of the number of mature erythrocytes. This apparently was due to a depression of their formation rather than to increased destruction. No evidences of the latter were found in the marrow or the viscera. On the third to seventh day there was a marked relative increase of the number and often of the size of immature red and white blood cells. This was especially notable of the blast cells. The normal rise in granulopoiesis somewhat masked the true picture. Nevertheless, the proportion of myelocytes was abnormally high (figs. 2 and 3). From the seventh to fifteenth day pancytopenia developed. It progressed into total aplasia (fig. 4). Past the fifteenth day severe aplasia was prevalent. In this stage, there was progressive reduction of the fat vacuoles of the stroma, and the connective tissue became a myxomatous, "smudged" ground substance. This was not seen in the fat elsewhere. Instead, the usual changes of inanition were observed. Macrophages containing numerous iron-staining granules were seen in the marrow, the spleen and the thymus but not in the liver. There was polychromasia of the young red blood cell but no anisocytosis or poikilocytosis. The wide range of the size of the normal fowl erythrocyte makes this judgment unreliable. Mitotic activity, particularly prominent in erythropoiesis, was not reduced except in advanced aplasia. There was no extramedullary hemopoiesis. The birds which received a folic acid–free diet supplemented with folic acid throughout the time of the experiment showed no abnormalities except for a moderately increased proportion of normal-appearing erythroblasts.

Bowel.—The earliest and most impressive departures from the normal were seen in the small bowel, especially in the distal third (fig. 5). On the third to fifth day the cells of the glands swelled. The nucleus became vesicular, the cytoplasm lighter staining and the cell margin indistinct. At times the cells appeared multinucleated. Mitotic activity was apparently not impaired (fig. 6). Somewhat later (five to ten days) the changes described were accentuated. The configuration of the cells at the base of the gland was greatly disturbed. The lumen of the gland was narrowed. From the tenth day on, numerous retention cysts, filled with a deeply staining mucoid material, appeared. Still later there was atrophy of the mucosa, characterized by a narrowing and by an increase of the fibrous elements of the stroma (fig. 6). At no time was ulceration of the small and large bowel seen. When the aforementioned changes were most severe, the esophageal, colic and occasionally lingual mucosa but not the gastric (proventriculus and ventriculus), showed the same abnormalities, especially at the base. In 2 instances only were there frank erosion and ulceration of the mucosa of the crop. In these cases the mucosal changes were most severe.

Other Organs.—No appreciable abnormalities were seen in the mucosa of the respiratory, biliary, pancreatic, urinary or genital tracts. No significant changes were seen in the other viscera or the musculature. The skin and its appendages appeared normal throughout. This may be due to the short time of the experiment. Late in the experiment the thymus, the spleen and the lower part of the bowel had slightly less than the normal number of mature lymphocytes. In the thymus the corpuscles of Hassall were larger and more numerous than

usual for the given age of the bird. The brain showed no degenerative changes, but the series of specimens from this organ was not as complete.

Although the feces of the starved animals were fluid, sections of the bowel showed general atrophy of all cell elements; in none, however, was there evidence of the changes seen in the folic acid–deficient or the antagonist-treated birds. On the other hand, at the end of ten days of malnutrition the bone marrow was markedly hypoplastic and its stroma myxomatous. At no time were

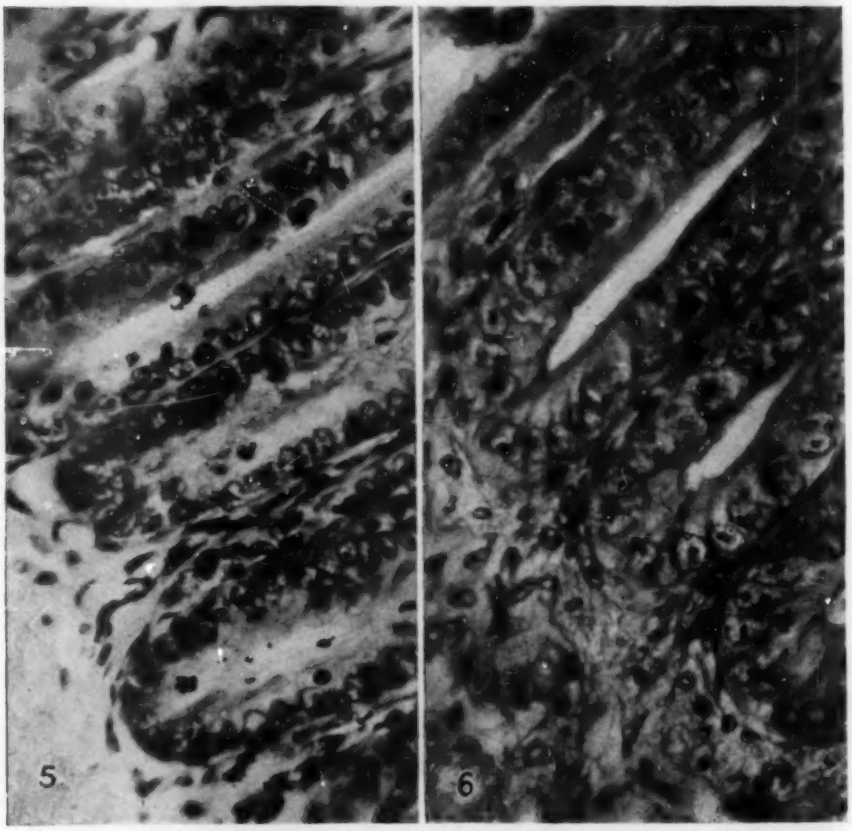

Fig. 5.—Duodenum of a normal 15 day old chick. Hematoxylin and eosin; × 650.

Fig. 6.—Duodenum of 15 day old chick restricted to a folic acid–free diet. There are marked swelling of the cells, with vesiculation and swelling of the nuclei and concentration of chromatin, and disordered configuration of the cells. Several mitotic figures are seen. Hematoxylin and eosin; × 650.

rubriblasts or prorubricytes or abnormally numerous immature cells seen; nor was there any hemosiderosis

Of particular interest is the finding that the early changes in the marrow and bowel were seen in a group of animals treated with a large dose of the antagonist for two days only. The birds treated with folic acid after ten days of deficiency showed strikingly rapid recovery of the marrow (two to three days) and

comparatively delayed recovery (five days) of the bowel. However, the marrow was not hyperplastic. Recovery appeared to be a recapitulation of the described pathologic changes in reverse.

COMMENT

The findings in the bone marrow are in accord with previous reports. As to those in the bowel, the work of Rinehart and Greenberg is generally supported, although a detailed description of their findings is lacking at the time of this report. The resemblance of the lesions seen in this study to those of the sprue is striking. It is generally held that the bowel changes in this disease are nonspecific and secondary to general malnutrition. This study supports the view that although the intestinal lesions of sprue are seen in advanced cases, they are not altogether nonspecific, for in our study early cytologic alterations were observed after only two days of treatment with a large dose of the antagonist and before appreciable malnutrition had developed.

The fact that administration of an oral antagonist and maintenance on a regular diet were associated with relatively little evidence of deficiency in the older birds is possibly related to the observations of Little and co-workers [6a] that there is a direct quantitative relationship between the age and weight of the animal and the amount of antagonist necessary to produce a maximal effect. They observed that, within physiologic limits, a larger amount of antagonist was necessary to inhibit the Rous sarcoma in the older and heavier bird. Other possibilities are a change in the flora of the intestinal tract or a reduced requirement for folic acid in the older animal.

This and the earlier cited investigations support the view that folic acid deficiency, induced by diet or by antagonist, has, from a morphologic standpoint, a maximal effect on the bone marrow and the gastrointestinal tract, especially the distal third of the small bowel. In neoplasia the effect is even more pronounced than that on the structures named. Apparently, therefore, the various cells of the body need folic acid for their metabolism in various degrees, and this substance is particularly necessary for the cells of a cancer. With the use of a suitable antagonist the metabolism of a cancer cell is thus especially, but not exclusively, interfered with.

SUMMARY AND CONCLUSIONS

Folic acid deficiency was produced in chicks by feeding a folic acid–free diet or by administering a folic acid antagonist to them. The animals were killed at frequent intervals and the viscera, bone marrow, brain, skin and skeletal muscle examined grossly and microscopically.

Folic acid deficiency of chicks is characterized by: (*a*) a rubriblastic, anemic and granulopenic marrow, the deterioration of which

progresses to a total aplasia with slight hemosiderosis; (*b*) degeneration of the glandular epithelium of the small and large bowel and, to a lesser extent, of the mucosa of the esophagus and the tongue, characterized by swelling of the cells and their nuclei, loss of normal cell configuration, gland retention cysts and ultimately atrophy.

Identical lesions are produced by a folic acid antagonist, such as 4-aminopteroylaspartic acid.

In the older animal the antagonist-induced deficiency is not so impressive.

The possible implications of the observations with respect to tropical sprue and Rous sarcoma are discussed.

University of Vermont College of Medicine.

General Reviews

SPONTANEOUS DEMYELINATING DISEASES OF ANIMALS

A Study in Comparative Pathology

LESTER S. KING, M.D.
AND
MARJORIE C. MEEHAN, M.D.
CHICAGO

A GREAT variety of spontaneous disorders of the nervous system exists in lower animals. Some of these have received considerable attention from both the descriptive and the experimental standpoint; to others but little attention has been paid. Apart from any economic advantage, the study of animal diseases is of value from a double standpoint—first, as disclosing fundamental pathologic relationships in natural hosts; second, as casting light on human disease. In general, pathologists have been especially interested from the latter standpoint.

It is the purpose of this communication to review in critical fashion certain spontaneous diseases of the nervous system in animals which are relevant to the problems of demyelination found in man and to discuss some of the general pathologic considerations raised thereby.

DEMYELINATION IN MONKEYS

A spontaneously occurring demyelinating disease of apes and monkeys has been reported repeatedly in the European literature of the present century. Although the descriptions of clinical symptoms and pathologic changes have varied in minor details, and the interpretations of the nature of the conditions have not all agreed, there seems little doubt that most or all of the reports refer to the same disease. Lesions may occur in the white matter of the hemispheres, the optic nerves and tracts, the spinal cord or in any combination of these.

Rothmann [1] in 1906 described a case which he called a "tabes-like disorder of apes." A rhesus monkey which had not been subjected to any experimental procedures was observed to have limited vision and slow, clumsy movements. Neurologic examination showed diminution of patellar reflexes, diminished sensation in the limbs and unsureness of movements. Despite these symptoms, the monkey was

From the Pathological Laboratory and Psychiatric Service, Illinois Masonic Hospital.

1. Rothmann, M.: Monatschr. f. Psychiat. **20**:204, 1906.

used for experimental purposes. The decussation of the pyramids of the medulla oblongata was sectioned, and later the arm area of the cortex was extirpated bilaterally. The animal died three months after the first operation. Postmortem examination of the spinal cord and the optic chiasm—no mention is made of other parts of the brain—revealed an area of degeneration of the posterior columns varying somewhat in extent at different levels of the cord but most marked in the thoracic region. At all levels it was most noticeable around the posterior septum and in the peripheral (dorsal) portion of the columns. Except for demyelination, the changes were not clearly described. Demyelination was also discovered in the optic chiasm. Rothmann considered the condition strongly suggestive of tabes but was reluctant to identify it as tabes because of the difference in distribution of the lesions—most marked in the dorsal region. No later writer has accepted this disease as tabes.

In 1908 Schröder [2] reported an excellent clinical and pathologic study of a spontaneous disease in Cercopithecus fulginosus which he thought might be an earlier stage of the condition described by Rothmann. In 1919 Kuhn and Steiner [3] and Steiner [4] described a monkey which had received by intra-abdominal injection 1 cc. of spinal fluid from a patient with multiple sclerosis. Eleven months later weakness of the hindlimbs was observed, but it cleared up quickly. Fifteen months after the injection, spastic paralysis appeared, and after four weeks' observation the animal was killed and examined. In the white matter of both hemispheres were many foci, some confluent, of demyelination, in most of which some axis-cylinders remained. Glial fibers were numerous, and at the periphery of the lesions there were many lipophages (*Körnchenzellen*) and much fat. Kuhn and Steiner considered the condition typical of multiple sclerosis, probably induced experimentally, but they admitted the possibility of spontaneous disease. Later writers have generally agreed that this case should be included among the spontaneous cases. Davison [5] stated that Steiner himself later reached this conclusion. No further examples have been found in the literature until 1930. But since then more than 40 cases have been carefully studied in continental Europe and England, and reference has been made to many other animals sick with apparently the same symptoms.[6] In 1944 Scherer [7] published a thorough review of all reported cases, including 20 which he had personally studied.

2. Schröder, P.: Arch. f. Psychiat. **44**:193, 1908.

3. Kuhn, P., and Steiner, G.: München. med. Wchnschr. **66**:1245, 1919.

4. Steiner, G.: Ztschr. f. d. ges. Neurol. u. Psychiat. **17**:491, 1919.

5. Davison, G.: J. Neurol. & Psychopath **14**:227, 1933.

6. (*a*) Perdrau, J. R.: J. Path. & Bact. **33**:991, 1930. (*b*) Levaditi, C.; Lepine, P., and Schoen, R.: Compt. rend. Soc. de biol. **104**:986, 1930. (*c*)

Although the greatest number of affected animals have been rhesus monkeys, most primates seem to be susceptible, the disease having been reported observed in orang-utans, baboons, various types of monkey and a lemur. It has not been found in any of the platyrrhine monkeys, chimpanzees or gorillas. Sex is not a factor. The age distribution, obviously, is not known accurately, but most or all of the subjects were adults. Although not all the accounts mention the origin of the animals, none state that these were born in captivity. Several of the animals were known to have been captured in Sumatra; others in Simla. It is not known whether the disease occurs among animals in their native state, since all the studies have been made in zoos or scientific laboratories. The case described by Hurst and McLennon [6q] occurred in an open-air zoo with conditions comparable to the natural environment.

The clinical picture is somewhat variable. Usually peculiarities of gait, clumsiness of movement and weakness of the limbs were the earliest signs, followed by increasing ataxia and partial paralysis. Some of these animals showed visual defects up to complete blinding. There is evidently much uncertainty whether sensory changes occur. In another group of animals, blindness was the first, and sometimes the only, symptom. In at least 1 case no clinical neurologic signs were observed; the animal died of tuberculosis, and the typical lesions of the brain were an unexpected finding in a routine autopsy.[6m] The animals usually lived from one to six months after the onset of symptoms, and death often occurred from intercurrent disease, frequently tuberculosis.

Although most of the cases were carefully studied, there were no constant significant findings outside the nervous system. Only once was any abnormality of the meninges noted—when Gärtner [6f] described a few lipophages in the pia mater overlying a lesion of the spinal cord. No changes have been discovered in any portions of the gray matter, cortex, basal ganglions or central gray matter of the cord. The lesions were confined to the white matter of the cerebral hemispheres, the

Schob, F.: Ztschr. f. d. ges. Neurol. u. Psychiat. **135**:95, 1931. (*d*) Scherer, H.: ibid. **141**:212, 1932. (*e*) Gärtner, W.: Klin. Wchnschr. **11**:905, 1932; (*f*) Arch. f. Psychiat. **99**:822, 1933. (*g*) Levaditi, C.; Hornus, G., and Schoen, R.: Compt. rend Soc. de biol. **113**:288, 1933; (*h*) Bull. Acad. de méd., Paris **110**:394 and (*i*) 406, 1933. (*j*) Hamerton, A. E.: Proc. Zool. Soc., London **104**:389, 1934; (*k*) **106**:659, 1936; (*l*) **107**:443, 1937. (*m*) Scherer, H. J.: Rev. neurol. **68**:897, 1937. (*n*) Hamerton, A. E.: Proc. Zool. Soc., London, s.B **108**:489, 1938; (*o*) **111**:151, 1941. (*p*) Von Bogaert, L.: Ann. Inst. Pasteur **63**:315, 1939. (*q*) Hurst, E. W., and McLennon, G. C.: M. J. Australia **2**:661, 1941. Davison.[5]

7. Scherer, H. J.: Vergleichende Pathologie des Nervensystem der Säugetiere, Leipzig, Georg Thieme, 1944.

optic nerves and tracts and the posterior and lateral columns of the spinal cord. The cerebellum and the corpus callosum were never involved. In the cerebrum there was nearly always sparing of the U fibers. Although in the majority of cases lesions occurred in all three of the typical locations—hemispheres, optic nerves and spinal cord—in several cases the cord was spared, while in others it was either not examined or not described. Again, many reports lacked descriptions of the optic system. The hemispheres were evidently normal in Rothmann's case and in some later cases.

In the hemispheres the lesions consisted of bilaterally symmetric areas of demyelination which in some cases were disseminated and in others diffuse. Authors describing widespread, or diffuse, areas generally agreed that they were made up by confluence of smaller foci. The foci might be round or irregular in shape. They were usually most numerous centrally and smaller and less numerous near the cortex, not involving the U fibers, except in a few instances. The lesions were most frequent in the parietal lobes, but common in the frontal and the occipital. Scherer [7] expressed the belief that the disease usually started in the parietal lobes and extended gradually forward and backward to the frontal and occipital areas. No foci have been described in the temporal lobes or orbital convolutions. Scherer stated that they were common in the upper third of the internal capsule. No plaques were found in the corpus callosum, but secondary degeneration of its fibers may be observed. Many of the lesions appeared to be perivascular. Demyelination was usually complete near the center of the foci, but sometimes there was a surrounding area of partial demyelination, shown by pallor in myelin stains. There is some difference of opinion as to the relative preservation of axis-cylinders, which usually appeared diminished in number and frequently damaged. Scherer stated that in the oldest lesions the axis-cylinders appeared better preserved than the myelin sheaths. In the earliest lesions there was some macroglial proliferation, followed later by microglial increase and the appearance of many lipophages. Except in the oldest lesions, there was usually much fat. "Gemästate glia cells" were frequent, especially around the vessels. In a few instances some perivascular round cell infiltration has been noted, but this was uncommon and seen only in older lesions. The vessels within the lesions showed no significant changes except some dilatation.

Lesions in the optic nerves, tracts and chiasm were frequent, but the descriptions are variable. Scherer [7] found 4 cases in which careful study revealed no abnormality in these areas. Schröder [2] described demyelination not in plaques but along the course of nerve bundles, although it was of varying intensity even in the length of a given bundle. Levaditi, Hornus and Schoen [6b] described demyelination in

plaques, with thickening of the vessel walls and perivascular collections of monocytes. Gärtner [6f] found demyelination in the temporal half of the optic nerves. Davison [5] considered the lesions in the chiasm and optic tracts similar to those in the hemispheres except for the absence of "gemästate glia cells." Scherer expressed the belief that the most constant finding is papillomacular degeneration, sometimes complicated by plaquelike foci.

Changes were present in the spinal cord in the majority of cases, although absent in a few carefully examined cases and not mentioned in others, in which the cord was evidently not examined. Most authors have described demyelination of the posterior columns, accompanied by some loss of axis-cylinders and the presence of fat-containing cells. This usually occurred in a roughly triangular area, affecting the more dorsal portions of the posterior columns at all levels of the cord except the lower lumbar and sacral. Frequently similar, though less severe, degeneration of the pyramidal tracts has been described. Scherer,[7] however, pointed out that the lesions of the cord are of two types—"funicular foci" of demyelination with numerous gitter cells and status spongiosus with minimal tendency toward glial scarring, and secondary degeneration of tracts elsewhere affected by these foci. The plaques were most common in the thoracic region and may occur, in posterior, lateral or anterior columns. Secondary degeneration may be present in cervical and lumbar regions. The plaques did not necessarily include the entire cross section of a given tract, nor were they always limited to single tracts.

Few studies have been made of the nerve roots and peripheral nerves. Gärtner [6f] described swelling of myelin sheaths and many lipophages in the sciatic nerves. Schob [6c] mentioned degeneration of posterior roots. Levaditi, Hornus and Schoen [6h] found satellitosis and neuronophagia, but no demyelination, in the spinal ganglions. Scherer [7] carefully studied the roots and peripheral nerves in all his cases and only rarely found changes—noninflammatory fascicular degeneration of posterior roots and sciatic nerve. He also studied the roots and nerves in many other monkeys and never found these changes in the absence of the disease under discussion.

The cause, the nomenclature and the extent to which the disease is related to various human diseases have occasioned much difference of opinion. Rothmann [1] suggested that it might be a form of tabes, but later workers have denied the possibility of syphilis. Schröder [2] considered it not identical with any human disease and merely conjectured that it was caused by some injurious substance acting on the myelin. Steiner,[4] as previously noted, considered it identical with multiple sclerosis, while Perdrau [6a] thought it the same as Schilder's disease (progressive subcortical encephalopathy). Schob compared it to both

of these but decided that it was identical with neither and merely described it as a spontaneous disease of unknown origin. Davison [5] agreed with this point of view, though suggesting the possibility of a disorder of lipoid metabolism.

Funicular myelosis, or subacute combined degeneration (dorsolateral spinal degeneration), is the human disorder with which this primate disease has been identified by Scherer,[8] Von Bogaert [6p] and Hamerton.[9] They pointed out the similarity of the spinal lesions and the fact that cerebral lesions have been frequently described in man, although much less often than in the monkey. Studies of the blood of monkeys have been made infrequently and when made did not show the findings of pernicious anemia.

In only 3 instances [6n] were stomach contents examined for hydrochloric acid. Achlorhydria was found in all 3. Nutritional disorders have frequently been noted. Hamerton [6n] apparently achieved cure in 1 case in which the clinical symptoms were typical, by injections of a purified liver solution (campolon®). Scherer [7] produced marked clinical improvement in 1 case by improving the diet. Although the authors admitted that these isolated cases may represent spontaneous remissions, they expressed the belief that the evidence strongly suggests nutritional deficiency as the cause.

The possibility of an infectious basis has been stressed by Gärtner [6e, f] and by Levaditi and his colleagues,[6g, h] who mentioned the epidemiologic evidence. A group of orang-utans was brought to Europe from Sumatra. Many of these died, including the one observed by Schob [6c] and others with similar symptoms at Halle, Germany. Later a similar disease developed in a Cynopithecus maurus in a cage near the sick orang-utans, and still later 2 baboons housed nearby showed the same symptoms. Levaditi, Hornus and Schoen [6g] described a group of 120 monkeys brought from Simla, capital of Delhi province, India, to Milan, Italy, in 1932, 12 of which became sick with presumably the same condition. Four of Scherer's animals were members of a group of 10, all of which got sick on the same transport.

Levaditi, Hornus and Schoen [6l] attempted to transmit the disease experimentally. They prepared an emulsion of the brain of a monkey with typical findings and injected it intracerebrally into a chimpanzee, a Cynocephalus babuin, a Cercopithecus callithrix, a Macacus cynomolgus, and 2 rhesus monkeys. No positive changes occurred in any of these except 1 rhesus monkey which four months after the inoculation became weak and hyperexcitable to touch and died one month later. At autopsy hemorrhagic foci were found in the basal ganglions

8. Footnotes *6d*, *6m* and *7*.

9. Footnotes *6j*, *6k*, *6l*, *6n* and *6o*.

and the choroid plexus and at the base of the brain. The lesions were of two types—acute lesions marked by thrombosed vessels, polymorphonuclear leukocytes infiltrating the vessel walls, some necrosis and numerous lipophages, and chronic lesions with demyelination and increased oligodendroglia and cells containing fat granules. They considered this a successful transmission experiment and suggested that the other experimental animals failed to contract the disease because they were not of the same species as the original source of the infective material. However, since both the clinical and the pathologic evidences of this disorder were quite different from those of the spontaneous disease, we do not believe that this can be considered evidence of transmissibility. Gärtner [6e, f] also attempted to transmit the disease. He injected spinal fluid from a sick animal intramuscularly into a rhesus monkey, in which in a few weeks typical symptoms developed, and at autopsy areas of destruction of axis-cylinders and increase of astrocytes, with no inflammatory changes, were demonstrated. He mentioned making a second successful passage from the experimental animal to another. Unfortunately, Gärtner's work is reported in two brief papers, lacking in detail, and we can find no evidence of its ever having been repeated or confirmed. We do not believe there is any convincing evidence that the disease can be transmitted. The evidence of at least a pseudo epizootic occurrence might have an infectious origin or might be related to dietary conditions.

In summary, a wide variety of apes and monkeys have been affected by a demyelinizing condition affecting brain, optic nerve and tracts, and spinal cord, of subacute to chronic course. In spite of diversity of pathologic description, all the reported animals appear to be suffering from the same disease entity. The distribution of cases suggests an epizootic nature of the disease, but no infectious or transmissible agent has ever been satisfactorily demonstrated. Dietary or other environmental factors are suggested as etiologic but have not been proved to be the cause.

SWAY-BACK

A nervous disease of newborn and suckling lambs has been noted in various parts of the world since, perhaps, as long as one hundred years ago.[10] It has been known by various popular names—sway-back, swing-back, *warfa, renguera,* Gin Gin rickets—but was not studied scientifically until recent years, when it became a serious economic problem.

In 1917 Gaiger [11] published a study of *renguera* occurring in the Sierra region of Peru. The name of the disease was said to be derived

10. Innes, J. R. M.: Personal communication to the authors.
11. Gaiger, S. H.: J. Comp. Path. & Therap. **30**:185, 1917.

from words meaning "broken back." He stated that young lambs aged from 6 weeks to several months were affected, showing weakness and incoordination of the hindlimbs, sometimes tremors of the head. Death usually ensued. He found no constant autopsy results except some increase in the color of the meninges, but apparently his pathologic examinations were casual and incomplete. There was evidence suggesting that a micrococcus might be the cause, but this was not confirmed by subsequent work.

In 1920 Magnusson [12] described a disease occurring in nursing lambs in Sweden with similar symptoms. He mentioned a few cases which had been noted elsewhere and expressed the belief that the condition might be the same as *renguera*. He, too, failed to find significant pathologic changes. Transmission experiments were unsuccessful. Innes [10] suggested that this may not be the same disease, since no subsequent cases have been observed in Sweden.

In 1932 two significant papers appeared. Bennetts [13] described what he called enzootic ataxia, which had been noted in certain parts of Australia for twenty years. It occurred in lambs from 6 weeks to 4 months of age and was characterized by ataxia and malnutrition, leading to death. Farmers had noted the disease only in lambs born of ewes which had pastured in certain affected areas during pregnancy. The disease could be prevented by sending the ewes to a different locality for part of their pregnancy. Bennetts found no lesions in the brain but observed consistent changes in the spinal cord following Marchi stains. He stated that there was "definite degeneration of certain nerve fibres in the thoracic and lumbar spinal cord" and that "scattered fibres in the femoral and sciatic nerve and its branches were also degenerated." In some cases the disease ran a prolonged clinical course, and in these cases he observed definite "sclerosis" of the cord.

In the same year Stewart [14] wrote a paper on "swing-back," which he stated occurred all over Britain but especially in southern Scotland and northern England. He also described the incoordination of the hindlimbs, which he said was made worse by excitement. The mildly affected lambs could walk a short distance but when driven farther would fall exhausted. If left alone, they would soon be able to get up and advance another short distance. If the condition appeared in one of twins, the other twin was certain to show the disease also. Stewart distinguished two types of the disorder. In the congenital form symptoms appeared shortly after birth; the lambs were unable to rise or suckle and soon died. The chronic type, which more closely

12. Magnusson, H.: Deutsche tierärztl. Wchnschr. **28**:297, 1920.
13. Bennetts, H. W.: Australian Vet. J. **8**:137 and 183, 1932.
14. Stewart, W. L.: Vet. J. **88**:133, 1932.

resembled the condition described by Gaiger and by Bennetts, appeared when the lambs were somewhat older—10 to 14 days—and had a longer course. He described tracts of degenerated fibers in the medulla and cord, especially the thoracic part of the cord. He believed that the affected portions were homologous to the direct and ventrocerebellar tracts and the column of Goll (fasciculus gracilis). He also mentioned an "abnormal condition of the cells of Clarke's column" at the thoracic level.

Between 1937 and 1944 a number of important contributions were made by Innes and co-workers [15] in England, who firmly and for the first time established the pathologic foundation, and by Bennetts and his colleagues [16] in Australia. The disease as observed in England appeared more acute than the Peruvian and Australian forms, occurring in newborn or very young lambs. Innes and Shearer [15c] noted lesions of the brain rather consistently in their cases. In the most severe form there was symmetric gelatinous degeneration of the white matter of the hemispheres, frequently with cavitation. In the earlier stages a type of demyelination occurred, which was compared to Schilder's disease. Histologically, the demyelination occurred rapidly, and axis-cylinders were destroyed as quickly as myelin. There was not much accumulation of stainable lipoids, nor any marked perivascular cellular infiltration. No inclusion bodies, neuronophagia or evidences of inflammation were found. The nerve cells were not damaged except for chromatolysis in the red nucleus, which these authors considered related to the lesions of the spinal cord. Where cavities occurred, the walls were composed of glia fibers, and fibrous astrocytes were noted. There was not much evidence of microglial participation. Degeneration of the motor tracts of the cord was noted. Innes concluded that the disease was of antenatal origin but that it must begin late in gestation. His studies showed the disease to be true degeneration of myelin and not aplasia. The tendency to liquefy was due to the characteristic reaction of infantile tissue. Culture and transmission studies repeatedly failed to give any evidence of an infectious etiologic agent. In healthy lambs which had been suckled by the mothers of sway-back lambs the disease did not develop.

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15. (*a*) Innes, J. R. M.: J. Neurol. & Psychiat. **2**:323, 1939; (*b*) Proc. Roy. Soc. Med. **33**:169, 1940. (*c*) Innes, J. R. M., and Shearer, G. D.: J. Comp. Path. & Therap. **53**:1, 1940. (*d*) Shearer, G. D.; Innes, J. R. M., and McDougall, E. I.: Vet. J. **96**:309, 1940. (*e*) Innes, J. R. M.: Vet. Rec. **55**:369, 1947. (*f*) Shearer, G. D., and McDougall, E. I.: J. Agric. Sc. **34**:207, 1944.

16. (*a*) Bennetts, H. W., and Chapman, F. E.: Australia Vet. J. **13**:138, 1937. (*b*) Bennetts, H. W., and Beck, A. B.: Bulletin 147, Council of Scientific and Industrial Research of Australia, 1942, p. 1.

The first clue to the etiologic explanation was announced by Bennetts and Chapman [16a] in 1937. Several investigators had suspected that lead might cause the condition because most of the areas where it occurred had relatively high concentrations of lead in the soil, although the diseased animals showed none of the usual findings of plumbism. In the hope that the animals might be "de-leaded" they were treated with ammonium chloride, and although at first the results seemed promising they were most inconsistent. Further investigation showed that the commercial preparation of ammonium chloride being used contained various impurities, including copper. Further studies revealed that chemically pure ammonium chloride was of no prophylactic or therapeutic value, but that if ewes were given extra copper, the disease was prevented in their lambs. In one experiment 12 ewes received a daily drench of copper sulfate. In none of their lambs did the ataxia develop. A control group of ewes was kept under similar conditions without the added copper, and the lambs showed a 100 per cent incidence of ataxia. These investigators also found that if the sick lambs were treated adequately with copper this disease was apparently cured. They noted macrocytic anemia in the pregnant ewes in the affected area and an abnormally low copper content in their liver, blood and milk. Similarly, the liver of the diseased lambs was low in copper. On the basis of their findings, Bennetts and Chapman suggested that there was a similarity between this disease and pernicious anemia, a concept which in view of later work does not seem to be valid.

Bennetts and Beck [16b] presented a longer and more detailed study in 1942. They noted that the disease was confined to the progeny of ewes which had been in the affected area at least six months and that the incidence of the disease increased the longer and more heavily a given region was stocked. Whereas in their previous Australian studies they observed that all the cases concerned lambs 6 weeks old or more and that none had lesions of the brain, they now reported several instances of a much more acute form of the disease. Lesions of the brain were found, with fluctuant softening and cavitation of the white matter. Four of these brains were examined by Innes, who agreed that the cases were similar to the English cases. In most animals degeneration of myelin occurred from the middorsal to the midlumbar regions of the spinal cord, particularly in areas which corresponded to the direct cerebellar tracts and Lissauer's tract (fasciculus dorsolateralis), but not definitely systematized. Damage of the gray matter was not found. Some scarring was observed in chronic cases.

Bennetts and Beck reported several further experiments indicating the etiologic role of copper deficiency. They showed that in the affected areas there was an abnormally low copper content of soil and herbage; that the ewes and affected lambs had levels of copper below the average,

and that the disorder might be prevented by supplying extra copper to the pregnant and lactating ewes and sometimes cured by giving it to the lambs. They also noted that the ewes with copper deficiency showed stringy wool and that the wool improved when extra copper was supplied. Anemia was frequent but not constant in the diseased animals.

Innes and co-workers [15] in several experiments confirmed the belief that copper was prophylactic and that in the blood and liver of the affected animals copper levels were low. In contrast to the findings in Australia, stringy wool was never noted in England. Further, there was no evidence that the copper content of the soil or herbage of those areas in England where the disease was most prevalent was unusually low. It was noted that ewes of Derbyshire, where the disease was common, if moved to Cambridge, where it did not occur, showed increase of copper and bore normal lambs, although repeated examination of the herbage in the two areas showed similar copper contents. Innes and his co-workers felt that the role of lead could not be entirely ruled out, possibly as affecting the utilization of copper. They also speculated on the significance of the calcium-phosphorus ratio. Innes [15a] published an interesting review of the studies to date in 1943.

McDonald,[17] in 1942, summarized the findings on this disease in Australia. In general, he supported the findings of the other workers and showed that the condition occurred in South Australia. He made autopsies in 22 cases, in 1 of which he found cavitation in the hemispheres, but in the others he noted changes only in the cord, except for the degenerative changes found in cells of the red nucleus. He described similar changes in the large multipolar cells of the ventral horns of the spinal cord and in the medulla. He noted that although anemia may be present, it is not an essential part of the syndrome, and he explained that sway-back often is complicated with a disease due to cobalt deficiency, which may result in anemia. He suggested that the difference in frequency of lesions of the brain as described by Innes and the Australian investigators is a reflection of a difference in intensity of the disease. He also described 1 case in which sway-back occurred in a 4 year old animal which had been under careful observation all its life. Ataxia developed, and one month later the animal was killed and examined. Typical demyelination and nerve cell changes were found.

Also in 1942 there appeared an extensive review of *renguera*, by Tabusso [18] in Peru. Neuropathologic studies confirmed the belief that

17. McDonald, I. W.: Australian Vet. J. **18**:165, 1942.

18. Tabusso, M. E.: Publ. Inst. nac. biol. anim., January 1942, vol. 1, no. 1; Vida Agri, October 1941 (This reference we have been unable to locate in any library, but through the courtesy of Dr. Innes a reprint was made available to us.)

it was identical with sway-back. He mentioned that copper had been shown to have prophylactic value but did not describe experiments and was not convinced of the etiologic importance of copper deficiency. He referred to the successful transmission experiments of Mitchell suggesting a virus as the cause, but this suggestion was not confirmed. Innes [15e] expressed doubt whether Mitchell was really dealing with *renguera*, and not with louping-ill.

In 1947, in an interesting paper,[19] it was reported that four of the seven investigators in England most active in these studies had subsequently suffered from nervous diseases resembling multiple sclerosis. This, of course, suggested the possibility of an infectious agent which acted only in the presence of a "trace element" deficiency. There has been no other evidence that sway-back was infectious. Transmission experiments have consistently failed to reproduce the disease, and there is no evidence of contagion. The paper in question, although of interest, does not rule out coincidence and adds no real proof of the infectious nature of sway-back.

In summary, sway-back is a disease of young lambs characterized clinically by ataxia and pathologically by demyelination in the spinal cord, chromatolysis of cells of the red nucleus and sometimes of the ventral horns, and, in the more acute cases, symmetric gelatinous softening leading to cavitation of the white matter of the hemispheres. There is convincing evidence that copper deficiency in the pregnant ewes plays an etiologic role and that the disease may be prevented by giving copper supplements to the ewes, but the mechanism by which the copper is effective is not known.

DEMYELINATION IN HORSES

A peculiar noninfectious nervous disease of horses has been observed by veterinarians from time to time since at least 1891.[20] Buckley and MacCallum [21] in 1901 and 1902 described what they called first "acute hemorrhagic encephalitis," later "acute epizootic leucoencephalitis," prevalent among horses in Maryland. They stated that, according to most observers, the disease occurred when horses were fed moldy fodder. Clinically there was some variation in symptoms, which included drowsiness, impairment of vision, paralysis of the pharynx, muscular twitching, unsteadiness of gait and weakness, followed by coma

19. Campbell, A. M. G.; Daniel, P.; Porter, R. M.; Ritchie, R. W.; Smith, H. V., and Innes, J. R. M.: Brain **70**:50, 1947.

20. (*a*) Mayo, N. S., in discussion on Schwarte.[24d] (*b*) Mizelle, J. D., and Graham, R.: Cornell Vet. **27**:374, 1937.

21. (*a*) Buckley, S. S., and MacCallum, W. G.: Am. Vet. Rev. **25**:99, 1901. (*b*) MacCallum, W. G., and Buckley, S. S.: J. Exper. Med. **6**:65, 1902.

or delirium. The course was rapid, death sometimes occurring within three hours after onset of symptoms, sometimes after as much as one week. The average duration was about three days. Most affected horses died. The few which recovered showed diminished intelligence or were "dummies." Buckley and MacCallum studied the brains of 4 animals which had died during the acute attack and on which autopsies were made; cultures of the brain material were negative. The meninges were slightly congested or normal, and there was no inflammatory exudate. The gray matter was always normal. In the white matter of the brain there were soft, fluctuating areas containing a grayish yellow pulpy mass of necrotic tissue and glairy opalescent fluid, mixed with dark blood. Adjacent to the cavities there were numerous hemorrhages, and microscopically there were loss of nerve tissue with persistence of glia cells and a moderate exudation of leukocytes. Axis-cylinders showed degeneration, swelling and thickening but persisted fairly well into the necrotic areas, where they terminated abruptly. The myelin sheaths were broken up into globules which took myelin stains. Disintegration of glia cells were described and the presence of a few fat granule cells noted. None of the large blood vessels were occluded, but the walls of many small vessels were infiltrated by leukocytes and round cells, and lumens were often occluded by hyaline thrombi. Buckley and MacCallum also made an autopsy of one of the recovered "dummies" and found a grayish translucent ramifying scar in the white matter of the anterior part of the cerebrum, which microscopically was composed of loose fibrous tissue.

In 1902 Butler [22] described the same disease and reported an experiment in which 2 healthy colts were fed moldy corn from a farm where 4 horses had died. One colt became ill twenty-six days after the experiment began and died in three hours. At autopsy the white matter of the left cerebral hemisphere was almost entirely broken down and the right hemisphere contained a small area of necrosis.

Dexler,[23] in 1903, published a study of hemorrhagic encephalitis of unknown origin occurring in horses in which there were disseminated areas of softening. It is impossible to determine whether he was discussing the same disease.

Although there was an outbreak of the disease in Illinois in 1914 and sporadic cases occurred in other corn belt states, not much further attention seems to have been paid to it until a serious epizootic occurred in 1934-1935, during which about 5,000 horses and mules died in Illinois and heavy losses occurred in Iowa. In the next few years a

22. Butler, T.: Am. Vet. Rev. **26**:748, 1902.

23. Dexler, H: Monatschr. f. Neurol. u. Psychiat. **13**:97 and 210, 1903.

number of papers dealing with this condition [24] appeared, mostly from Illinois and Iowa, and the following description is based on them.

The clinical syndrome varied in intensity. Graham and co-workers [24a, b] described three types—lethargic, nervous (or excited) and paralytic—and remarked that the clinical syndrome resembled that of virus encephalitis. Schwarte and co-workers [24c] also noted the similarity of this disease and virus encephalitis and pointed out that in the absence of autopsy the diagnoses are often confused. They mentioned as possible symptoms depression, incoordination, rolling on the ground, walking in circles and violent motor activity.

The autopsy findings outside the central nervous system were essentially without significance except that Schwarte and co-workers [24c] mentioned evidence of severe toxic changes in liver, kidneys and bladder. The brain showed softening or liquefaction unilaterally or bilaterally in the white matter. In the areas adjacent to the necrosis, edema and hemorrhages were noted, but no perivascular cuffing or round cell infiltration. Graham described degenerative changes of the nerve cells, and Schwarte mentioned some increase of glial elements. Occasionally microscopic hemorrhages, edema and degenerative changes occurred in the spinal cord.

All observers agreed that the disease seems to be related to the ingestion of moldy corn and that severe outbreaks are most likely to take place when weather conditions have been favorable for the growth of molds on the corn. Although often called "cornstalk disease," it appears that not only the stalks but the ears and the kernels may carry the causative agent. Experiments have been made without success to determine what this is. Butler's [22] experiment has been noted. Graham [24a] placed 8 horses in a stalk field where there had been previous occurrence of the disease. Two of the 8 horses died with more or less typical symptoms, and an autopsy of 1 horse was made, which showed the characteristic findings. Schwarte [24d] fed 5 horses moldy corn. Typical symptoms developed in all, and all showed the usual postmortem changes, while 3 control animals remained healthy. Biester and co-workers [24f] experimented with corn showing different types of mold, one predominantly green, the other gray, and with both produced some clinical symptoms and fairly typical autopsy findings. Feeding experiments were made with various molds isolated from corn and corn chaff

24. (*a*) Graham, R.: Vet. Med. **31**:46, 1936. (*b*) Graham, R., and Harris, B.: ibid. **31**:340, 1936. (*c*) Schwarte, L. H.; Biester, H. E., and Murray, C.: J. Am. Vet. M. A. **90**:76, 1937. (*d*) Schwarte, L. H.: ibid. **92**:152, 1938. (*e*) Biester, H. E., and Schwarte, L. H.: North Am. Vet. **20**:17, 1949. (*f*) Biester, H. E.; Schwarte, L. H., and Reddy, C. H.: Vet. Med. **35**:636, 1940. (*g*) Biester, H. E., and Schwarte, L. H.: ibid. **39**:303, 1944. Mizelle and Graham.[20b]

by Mizelle and Graham [20b] without positive results. All experimenters have agreed that it is impossible to isolate any virus or to transmit the disease by inoculation of brain. Graham and Harris isolated Pasteurella from 2 cases of the disease, naturally occurring, but there has been no further indication that these organisms play a causative role.

It has been noted that cattle and other animals are not made ill by the moldy corn which affects horses, but that in cattle a different disease may develop from consumption of other types of abnormal corn which are not harmful to horses.[25]

This disease has received little attention in neurologic or pathologic literature. Detailed neuropathologic and topographic analysis of lesions have not been carried out. Little has been added to the original pathologic description of MacCallum of 1902, in spite of the great advances in neuropathologic methods and technics since that time and the apparent great abundance of cases. Of present day neuropathologists, Hurst [26] alone appears to have had access to material. In general he confirmed the findings of MacCallum and Buckley.[21b] He noted that in the most severe portions of the lesions all nuclear staining was lost. Elsewhere early microglial reaction, with swelling and proliferation of adventitial cells and capillary endothelium, was observed. The central areas exhibited almost complete demyelination, with no axis-cylinders remaining. In the outer zones the myelin sheaths were varicose. Compared with lesions which he produced experimentally in monkeys by administering potassium cyanide, the lesions of the horses showed more complete central softening, a less abrupt transition from softening to a normal condition, and involvement of arcuate fibers.

Fundamentally the disease appears to be of toxic character, but the means by which the ingestion of the toxin is translated into catastrophic destruction of white matter is not clear. The pathologic changes, including hemorrhages, thrombi and presence of polymorphonuclear leukocytes, suggest a vascular mechanism. But the described changes do not resemble the picture associated with massive thrombosis, nor are the clinical and the experimental course of the disease consistent with cerebral thrombosis as commonly understood. The peculiar localizing of lesions to the white matter compels one to include this condition in the category of spontaneous demyelinizing diseases.

DEMYELINATION IN MOOSE

An obscure condition affecting moose has received sporadic attention in various fields of research. The relevant literature has been

25. Schwarte, L. H.; Eveleth, D. F., and Biester, H. E.: Vet. Med. **34**:648 1939.

26. Hurst, E. W.: Australian J. Exper. Biol. & M. Sc. **18**:201, 1940.

reviewed and the neuropathologic findings described by King.[27] The affected animals, observed in Maine and Minnesota, exhibited weakness, staggering, incoordination and drowsiness. There has been a trend toward seasonal incidence, with the majority of cases being observed between February and May, but sick animals have been found during other months as well.

It is probable that not all the observed sick animals were suffering from a single disease entity. King studied material from 3 animals of Maine, 2 of which showed similar lesions, while the third revealed none. Brain tissues from 5 sick animals of Minnesota were also studied, with positive results in 3 cases. Thus, of 8 cases examined, only 5 were positive for demyelination; in 4 of these characteristic changes were revealed, while the fifth was atypical. Fenstermacher and Jellison[28] are insistent that "the losses of moose that occur in Minnesota are not the result of a single pathogen."

It is not possible, therefore, to maintain that all the various studies of sick moose have dealt with the same disease entity. Experimental work carried out in cases in which demyelination was demonstrated has failed to exhibit any transmissible agent or to provide evidence of infection.

Pathologic examination showed damage of the white matter. Abundant neutral fat was observed in poorly defined foci. In some areas the fat was diffusely scattered through the involved tissues, while in others it had a predominantly perivascular distribution. Myelin stains showed zones of poorly circumscribed loss, with relative preservation of axis-cylinders. Fibrous gliosis was found. In the white matter of the cerebrum rather sharply defined perivascular scars were exhibited by appropriate glial stains. In the cerebellum and the medulla more diffuse gliosis was found, quite disproportionate to the amount of myelin lost. In the 4 typical cases cellular infiltrations were noted, virtually limited to the white matter and generally perivascular in distribution. The fifth case, with inflammatory involvement of the cortex, appeared atypical.

Examination showed evidence of a process of a relatively mild type, with destruction of myelin and fibrous gliosis, not sharply circumscribed, and accompanied in more acute phases by variable degrees of cellular infiltration. The cause is not known. All the available evidence points away from an infection and suggests the possibility of dietary deficiency or possibly the action of some toxic substance. This condition appears to be a disease entity responsible for the death of an unknown per-

27. King, L. S.: Am. J. Path. **15**:445, 1939.

28. Fenstermacher, R., and Jellison, W. L.: Diseases Affecting Moose, Bulletin, University of Minnesota Agricultural Experiment Station, 1933, p. 294.

centage of native moose. To our knowledge the disease has not been found in moose in captivity.

ENCEPHALOMYELITIS IN THE DOG

The problem of demyelination is more complex in the dog than in the animal species discussed hitherto. The demyelinations observed in monkeys, sheep, horses and moose represent, in all probability, single discrete disease entities. In the dog there is a variety of poorly defined diseases involving the nervous system. Demyelination is a feature of some but not of all of these involvements.

The confusion has been pointed out by Innes,[10] who distinguished six groups of nervous disorders in dogs, separable on pathologic, although not necessarily on purely clinical, grounds. These groups include (*a*) brains and cords without lesions, suggesting true canine hysteria, (*b*) inflammatory changes with demyelination, (*c*) vascular disturbances with blood vessel proliferation in the gray matter, (*d*) encephalitis with perivascular lymphocytic infiltration, (*e*) chronic lymphocytic meningitis and (*f*) true distemper virus infection. Unfortunately, in the literature most cases of cerebral involvement have been uncritically assumed to be instances of distemper virus infection and have been described as such.

Distemper is a specific disease entity, caused by a filterable virus. It is a highly infectious disease of dogs but may also, according to some authors, attack fox, wolf, hyena and monkey,[29] ferret, stoat and weasel.[30] Bacteria are recognized as secondary invaders. The encephalitozoon described by Kantorowicz and Lewy [31] is not etiologic. Clinically the disease shows a varied pattern of symptoms. In classic cases there is an initial rise of the temperature seven to eight days after exposure to infection, with recession and then a secondary rise, giving a diphasic fever. There is usually associated coryza, as well as frequent catarrhal symptoms of eyes and nose. Bronchitis is common, and bronchopneumonia may be present. Cutaneous lesions may occur varying from vesicular to pustular. Diarrhea and vomiting, or "gastro-intestinal catarrh," may be prominent. Inclusion bodies are of diagnostic importance, but their consideration lies outside the scope of this paper. One may refer to de Monbreun,[30] Green and Evans [32] and Watson and Plummer.[33] In some outbreaks nervous manifestations may appear.

29. Marinesco, G.; Dragenesco, S., and Stroesco, G.: Ann. Inst. Pasteur **51**: 215, 1933.

30. de Monbreun, W. A.: Am. J. Path. **13**:187, 1937.

31. Kantorowicz, R., and Lewy, F. H.: Arch. f. wissensch. u. prakt. Tierh. **49**: 137, 1923.

32. Green, R. G., and Evans, C. A.: Am. J. Hyg. **29**:73, 1939.

33. Watson, E. A., and Plummer, P. J. G.: Am. J. Vet. Research **3**:350, 1942.

According to Pugh, these nervous symptoms may occur weeks or months after the initial or primary symptoms, or may be seen without any of the primary systemic disturbance. According to de Monbreun, the nervous symptoms, if they occur, usually develop after the second week of the disease.

The clinical picture of distemper is so variable that veterinarians sometimes divide the disease into various types, catarrhal, intestinal, cutaneous or tegumentary, and nervous, depending on which system is most prominently affected.

All observers agree that the disease is prevalent throughout the world and that subclinical or atypical infections are frequent. So great is the variability that Dunkin and Laidlaw [34] declared it may be impossible to diagnose distemper (clinically) with certainty. To prove distemper it is necessary to rely not on clinical judgment but on biologic studies in which virus-containing materials are inoculated and protection tests made. Further difficulty is offered in the choice of a suitable test species. Dogs, unless very young, are not suitable, because they may have been immunized by previous subclinical infection. Ferrets, as demonstrated by Dunkin and Laidlaw, are the animals of choice. Thus, extreme practical difficulties attend the positive identification of this disease. It is probable that even skilled veterinarians are not entirely reliable in their clinical diagnosis of distemper. Certainly, in cases of the so-called atypical or abortive type the diagnosis cannot be accepted without rigorous proof. As Innes [15b] said, "The terms 'distemper' and 'post-distemper encephalitis' have been loosely applied and with no more proof than that the dog may, some time previously, have suffered from a febrile illness." We may say that the diagnosis of canine distemper bears the same relation to canine disease that the loose diagnosis of "flu" does to human febrile illnesses.

The most comprehensive pathologic description of the brain involved in alleged distemper was written by Cerletti [35] in 1912. Little of significance has been added by subsequent writers. Cerletti personally observed 32 dogs both clinically and pathologically; 28 of them had essentially "typical distemper"; in 10 it was clinically purely "catarrhal," while in 18 it assumed clinically the "nervous" form. The remaining 4 dogs showed nervous symptoms several months after their alleged distemper, and Cerletti admitted an assumption in relating these to the other groups. For our purposes these 4 dogs are inadmissible. Of the 10 dogs with the catarrhal form but without clinical nervous symptoms, 6 showed in the nervous system localized areas of intense plasma

34. Dunkin, G. W., and Laidlaw, P. P.: J. Comp. Path. & Therap. **39**:201 and 213, 1926.

35. Cerletti, U.: Arch. f. d. ges. Neurol. u. Psychiat. **9**:520, 1912.

cell and lymphocytic accumulation around pial vessels and infiltrating into nerve tissue, together with glial proliferation. These findings could not be qualitatively distinguished from those in the "nervous form." In the latter the inflammatory changes in the nervous system were more intense and widespread. Cerletti somewhat artificially distinguished three types of change, designated as focal (perivascular) infiltrations, "productive" foci characterized by proliferation of vascular adventitial and endothelial cells, and foci or nodules of glial proliferation. These occurred singly or in combination.

Today, with vastly increased knowledge of the pathologic aspects of virus encephalitides, these findings are entirely consistent with virus causation. With additions they have been confirmed by Hurst and co-workers,[36] who studied both the neuropathologic and the immunologic aspects.

It is of great interest that with regard to the inflamed areas Cerletti described and illustrated foci and areas of fat granule cells (*Körnchenzellen*). The legend of one of his illustrations, figure 10, shows the misprint *Körbchenzellen*. Beyond all reasonable doubt such zones were areas of demyelination such as were described later by Perdrau and Pugh [37] and others.

Cerletti's work, as mentioned in a foregoing paragraph, was in essence confirmed by Gallego [38] and others, who, however, did not evince any interest in the status of the myelin. Roman and Lapp [39] confirmed the fact that inflammatory lesions occur in the brain in the absence of clinical neurologic signs. None of these writers were critical of the accuracy of the diagnosis of distemper.

Perdrau and Pugh,[37] in 1930, reporting for the first time demyelination as observed in the dog, opened up a new era in canine neuropathology. They studied 14 animals, all suffering clinically from nervous disorder and showing pathologically disseminated encephalomyelitis, that is, scattered zones of inflammatory reaction involving the brain stem and spinal cord. Of these 14 animals, 7 had a history pointing to a previous attack of distemper. Of the total series, 3 from the distemper group and 1 of the remaining group showed demyelination as part of the disease process.

The authors pointed out that distemper has long been associated with encephalitis, known as "the nervous form of distemper," supposedly due to spreading of the virus to the brain. From the clinical standpoint they did not believe that in their cases a causal relation

36. Hurst, E. W.; Cooke, B. T., and Melvin, P.: Australian J. Exper. Biol. & M. Sc. **21**:115, 1943.

37. Perdrau, J. R., and Pugh, L. P.: J. Path. & Bact. **33**:79, 1930.

38. Gallego, A.: Ztschr. f. Infektionskr. **34**:38, 1928.

39. Roman, B., and Lapp, C. M.: Bull. Buffalo Gen. Hosp. **3**:40, 1925.

could be established between the virus disease and the demyelination. For example, one of their dogs, a puppy 8 months old, had always been well until it came in contact with an animal suffering from distemper. The puppy speedily became ill, exhibited nervous symptoms and was killed on the fourth day of illness. The brain showed chronic patches of demyelination, clearly older than the duration of the illness.

Perdrau and Pugh expressed the belief that "the encephalomyelitis commonly referred to as 'the nervous form of distemper' is not the result of the action of the specific virus of distemper on the central nervous system, but that the virus of distemper plays in this disease of the dog a similar role to that which an acute infection of varying etiology plays in the causation of certain demyelinizating diseases of man."

That the animals described by Perdrau and Pugh may not have been suffering from distemper received emphasis from Laidlaw,[40] who was unable to reproduce the disease in ferrets with brain tissue from some of the animals of Pugh.

Additional evidence concerning demyelination was speedily forthcoming. Marinesco and associates,[29] as well as Peters and Yamagiwa,[41] also described demyelinating changes of the brain in addition to inflammatory involvement. The loss of myelin occurred predominantly in the cerebellar peduncles, the medulla and the pons. Both of these authors claimed to be describing distemper encephalitis. But Marinesco and co-workers did not state the source of their material, and Peters and Yamagiwa did not exercise a sufficiently critical attitude in their analysis of their cases.

On the other hand, Scherer and Collet [42] emphasized the importance of skepticism in regard to distemper as a cause. They described 3 cases in which damage of the brain was observed, in dogs, all quite different. Only their second case was similar to those described in the foregoing paragraph, with disseminated foci of inflammatory change and demyelination involving pons, medulla, thalamus, hypothalamus, internal capsule and parietal white matter. The dog was sick for only three weeks, but the visceral organs showed no change. They expressed the belief that distemper was not the cause. Their third case, in which the clinical symptoms lasted for eighteen months, beginning one month after an attack of alleged distemper, is apparently unique in the literature. There was strictly perivascular inflammation,

40. Laidlaw, cited by de Monbreun.[30]

41. Peters, G., and Yamagiwa, S.: Arch. f. wissensch. u. prakt. Tierh. **70**:138, 1936.

42. Scherer, H. J., and Collet, L.: J. belge de neurol. et de psychiat. **39**:132, 1939.

with no infiltrations of tissue, but in addition hydrocephalus was present, with glial sclerosis involving the white matter of both hemispheres. This case they considered to be one of a peculiar post-distemper complication. But they emphasized that a histopathologic diagnosis of distemper cannot be made.

The first report of demyelination occurring in the dog to be published in this country was that of King.[43] The animal showed paralysis, spasticity, contractures and marked sensory loss. It had been ill for about three months. The lesions were those of disseminated encephalomyelitis, with widespread areas of severe inflammatory infiltrations in the parenchyma, involving all levels from spinal cord to cerebral cortex and optic tracts, but most severe in spinal cord, medulla, pons and cerebellum. In areas of severe myelitis, there was vigorus proliferation of reticulin fibers in addition to the destruction of white matter. In some foci of the cord, severe destruction of the gray matter was present, but ganglion cells were strikingly preserved. In the cerebellum and the medulla the lesions were discrete, generally with severe inflammation in the parenchyma. Different types of loss of myelin were noted, a feature which will be treated in the section with the heading "Comment."

King was unable to correlate the condition with distemper and described it merely as disseminated encephalomyelitis with demyelination.

Further data appeared in the literature which cast doubt on the association of demyelination and distemper. Findlay[44] quoted from an unavailable thesis of Verlinde to the effect that demyelinating lesions have been found in dogs known to be immune to distemper, but the work in question cannot be consulted. Innes[16b] referred briefly to 50 dogs that he studied. Their number was later considerably augmented, but all the anatomic preparations sent to Scherer and mentioned in his book, were lost during the war.[10] In his published note he stated conservatively that "more than one entity may occur in the dog which may have been regarded in the past as the nervous form of distemper . . . and in which the association of the distemper virus is problematical."

Scherer,[7] in his recently published book, stated that he did not believe in the direct relationship of distemper and the demyelinating lesions. He considered these lesions to be strictly comparable to acute multiple sclerosis in man, and he contended that this acute multiple sclerosis of dogs has nothing to do with distemper.

Certain experimental studies are of considerable importance. Posrednik[45] injected supposed distemper virus into 9 dogs and found brain changes indicative of meningitis and meningoencephalitis. But

43. King, L. S.: Arch. Path. **28**:151, 1939.
44. Findlay, G. M.: Proc. Roy. Soc. Med. **33**:161, 1939.
45. Posrednik, F. I.: Ztschr. f. Infektionskr. **38**:136, 1931.

no true demyelination occurred. In contrast, 3 dogs which had died of the natural disease showed severe inflammatory infiltrations with destruction of myelin of the type described by other workers. Posrednik emphasized that in severely inflamed zones there was always disappearance of myelin. Koch's postulates thus were not fulfilled. The severe brain damage and associated demyelination, or loss of myelin in any form, evidently require more than the mere presence of an infectious agent.

De Monbreun [30] studied the histopathologic aspects of natural and experimental distemper, paying special attention to inclusion bodies. With a single strain of virus he was able to produce the catarrhal form in some passages, the nervous form in others. In one animal, a puppy dying seventy-two days after inoculation, destructive demyelinating lesions were produced in the medulla and the pons. The photomicrographs are entirely convincing.

De Monbreun's work has been critized by Green and Evans,[32] who showed that the virus used in most of the transmission experiments was derived from an animal ill not with canine distemper but with fox encephalitis. This criticism is valid and indicates a difficulty facing research workers, namely, how to determine whether a given sick animal, even one whose brain contains an infectious transmissible agent, is actually suffering from distemper and not from some other disease. The utmost care, with cross protection and immunity tests, and additional use of ferrets, is necessary for accurate work.

For our purpose, however, the point at issue is whether demyelination can be produced by virus action. The problem whether the alleged causative agent is distemper, fox encephalitis or an unnamed virus is less important than that of demonstrating virus causation of some demyelinating conditions in some hosts. De Monbreun's case is not by itself conclusive. Other or accessory etiologic factors have not been excluded. But that a demyelinating lesion was experimentally reproduced with infectious material is an important fact.

A significant contribution is that of Hurst, Cooke and Melvin.[26] Hurst studied 9 dogs succumbing in two epidemics of distemper. Seven of the animals with nervous symptoms had suffered from the systemic disorder two or three weeks, 1 six weeks, previously. For 1 dog no history of previous distemper was elicited. The nervous illness lasted three to seven days and terminated fatally. The lesions observed in these brains were similar to those described by other workers, particularly Cerletti,[25] Perdrau and Pugh [27] and Marinesco and associates,[29] with parenchymatous and perivascular inflammatory changes and foci of demyelination. Using material derived from his demyelinated animals Hurst carried out extensive transmission experiments and protection tests, utilizing ferrets as well as dogs. For control

virus he used a strain of distemper virus obtained from the Commonwealth Serum Laboratories in Australia. He and his co-workers were able to show that the brains exhibiting demyelination and encephalitis contained distemper virus. Cross immunity tests were in general satisfactory, although results with a few dogs were not as satisfactory as might be desired. Hurst raised the question whether he was dealing with two closely related viruses. His conclusions are that the cause of "nervous distemper" is "the distemper virus itself, and that the demyelinating lesions produced by it represent damage to the white matter short of complete necrosis."

These positive results he obtained by employing biologic as well as histopathologic methods. Unless the two separated disciplines are employed together, the conclusions reached with either one alone are open to some question. The work of Hurst and co-workers does not solve the problem of the encephalopathy of dogs. His results indicate that at least in some dogs demyelination is caused by virus action, possibly distemper virus, although other viruses have not been definitely excluded.

The work of Hurst has been criticized by Innes,[10] who indicated that in all probability distemper virus was not being employed. According to the latter,[10] recent work by Macintyre, Trevan, and Montgomerie showed that several different infectious agents, with different properties, can be isolated from dogs ill with alleged "distemper." In his forthcoming paper, Innes discusses critically the problem of diagnosis of distemper and the relation of this to other virus diseases.

From our standpoint certain facts stand out. A wide variety of disorders of the nervous system occur in dogs. In the past there has been an unwarranted assumption that such disturbances may be designated as nervous distemper. This assumption must be actively combated. Nevertheless, many febrile infectious illnesses, with or without nervous symptoms, include meningoencephalitis or encephalomyelitis. In some of the dogs demyelination occurs, but demyelination is also seen in other dogs with encephalomyelitis that is unquestionably not distemper.

The common thread here is the presence of inflammation. Most of the foci of demyelination are areas of severe inflammatory change, as emphasized by Posrednik.[45] There is no inconsistency in holding that the inflammation produced by virus and nonvirus agents may show similar results.

A case in point is described by von Móczy,[46] who discussed the encephalomyelitis of dogs following rabies vaccination. Such animals

46. von Móczy, J.: Arch. f. wissensch. u. prakt. Tierh. **72**:15, 1938.

showed widespread severe inflammatory changes. One of his illustrations shows perivascular demyelination. Yet inoculation studies gave entirely negative results, and no transmissible agent could be demonstrated. Here one has demyelination in an inflammatory lesion that is apparently noninfectious but may well be on an allergy or sensitivity basis.

We must conclude with Hurst, Cooke and Melvin[36] that demyelination is not specific but is merely an indication of severe damage of tissue. This damage, reflected in inflammatory changes, may lead to loss of myelin. Destruction of myelin therefore is an index of damage to white matter, which unquestionably may have virus localization as one cause but which may have other causes as well. In the dog there is no positive evidence pointing to the nature of these other causes.

ENCEPHALOMYELITIS IN GOATS

The brains of goats suffering from an unusual infectious disease were studied by King.[47] Over a period of three months a series of 9 goats coming from a single farm in New Jersey all succumbed to an infection. The clinical symptoms lasted only two to three days, and were not distinctive. Specific neurologic signs were rare. The outbreak was studied by TenBroeck and Seastone,[48] who in 7 of the 9 cases recovered pure cultures of an organism belonging to the genus Listerella. Examination of brain tissues showed severe disseminated encephalomyelitis with lesions of predominantly granulomatous character restricted almost exclusively to the brain stem, especially the medulla and the spinal cord. In addition to severe focal parenchymatous nodules, marked perivascular cuffing was noted, and some areas of more diffuse infiltration of tissue. The bacteria could be demonstrated by Gram-Weigert stains in the tissue nodules and areas of infiltration. Investigation of the status of myelin revealed focal areas of destruction which, however, were not as widespread as might have been expected from the severity and extent of the inflammatory involvement. Axis-cylinders were better preserved in these areas than was the myelin. The fulminating course of the disease, however, prevented a full evaluation of the loss of the myelin.

The genus Listerella is responsible for a wide variety of infections in various domestic animals, including encephalitis as well as other tissue localizations. Encephalitis of sheep has been most commonly discussed. The status of myelin in such cases is problematic. It is claimed that the organism is responsible for some human infections. The literature is extensive and not entirely relevant to the present

47. King, L. S.: Am. J. Path. **16**:467, 1940.

48. TenBroeck, C., and Seastone, C. V.: Unpublished data; cited by King.[47]

discussion A satisfactory review has been published by Graham, Levine and Morrill,[49] and numerous articles regarding human and animal infections are cited by King.[47] A curious feature is that although in cases of animal encephalitis and encephalomyelitis the causative role of Listerella cannot be doubted, nevertheless Koch's postulates have not been fulfilled. Organisms can be demonstrated within the involved brain tissue, but pure cultures do not produce the natural disease. Fatal meningitis is readily produced but the characteristic disseminated parenchymatous involvement seen in the natural diseases has not been reproduced experimentally.

The significance of this condition is twofold. Loss of myelin is demonstrated in an acute natural infection, although the amount and the severity of the loss are not great and demyelination is not the ruling pathologic feature. Further, the damage of myelin is related directly to the primary inflammatory process and appears to be due to the direct localization of the infecting agent. These points will be considered in more detail in the comment.

MISCELLANEOUS OBSERVATIONS

A wide variety of diseases of the nervous system has been described as occurring spontaneously in lower animals. Many have been well studied; others, only sketchily described. Unfortunately, only rarely in studies on brain diseases in animals has the problem of the destruction of myelin received attention.

In our survey of the literature, we have encountered a few reports in which data are incomplete and which do not admit of any discussion. The following instances, however, deserve mention.

Scherer[7] mentioned briefly a demyelinating condition which he observed in bears but did not describe the disease at adequate length. In 2 animals he observed disseminated focal encephalomyelitis. There was absence of "diffuse inflammation," but small foci of lymphocytic perivascular inflammation, with loose macroglial and microglial proliferation, were noted. The lesions did not respect any boundaries between gray matter and white matter. Demyelination is mentioned as incomplete, but adequate details are not given.

Hamerton[63] described a middle-aged leopard with gradually increasing spastic paralysis of the hindlegs, becoming flaccid shortly before death. In the spinal cord, there was degeneration of pyramidal and cerebellar tracts and of the column of Goll, and the myelin was replaced by scarlet-staining globules of fat. There were disappearance of axis-cylinders, with neuroglial replacement, and "hardly any

49. Graham, R.; Levine, N. D., and Morrill, C. C.: Listerellosis in Domestic Animals, Bulletin 499, University of Illinois, Agricultural Experiment Station, 1943.

inflammatory reaction." He could not define the disease in terms of human pathology. His paper gives convincing illustrations.

In 1946 Hamerton [50] noted a tiger bittern which died of encephalitis. The brain showed "round cell infiltration along the course of the cerebral vessels and areas of demyelination in the intervening tissue." Further data are not presented.

COMMENT

Meaning of "Demyelinating Disease."—From the standpoint of nosology it is perhaps unfortunate that the myelin stain was ever devised. With its use, investigators can make magnificent histologic preparations exhibiting loss of myelin in a wide variety of different conditions. But emphasis has been placed on histologic details rather than on disease processes. If we wish to be literal, any disorder in which myelin is lost is a "demyelinizing disease." Yet common usage revolts at such catholicity and insists that certain diseases showing loss of myelin be categorized differently from others in which the destruction of myelin is also a prominent feature. With literal interpretation, using a myelin stain as the criterion, we should have to include such diverse states as wallerian degeneration, amyotrophic lateral sclerosis, brain abscess, Marchiafava disease and experimental cyanide poisoning, to name but a few.

It is desirable to distinguish between demyelination as a condition and demyelination as a disease. In the former the loss of myelin is secondary to some other pathogenic features. Thus, if cell bodies are first attacked, there results a loss of associated nerve fibers as a secondary phenomenon. By a similar pathogenesis interruption of axons leads to loss of the distal segments. Again, any destructive process which attacks white matter merely as an accident of location should also be excluded from "demyelinating diseases." The localization of an embolus, a thrombus, staphylococci or tubercle bacilli may be such as to produce widespread destruction of white matter. No one would call an infarct, an abscess, a traumatic cicatrix or a tuberculoma a "demyelinating disease," no matter how beautiful the Weigert stain of sections. In these instances the pathogenic factor attacks the white matter merely accidentally, by virtue of fortuitous localization, not by virtue of any essential relationship borne by myelin as such.

If one wishes to use the term "demyelinating disease," the chacterizing feature by contrast is that the disease process attacks primarily and essentially the white matter of the brain. The degree of preservation of axis-cylinders is in no sense fundamental. In most of the commonly accepted "demyelinating diseases" axis-cylinders may be well

50. Hamerton, A. E.: Proc. Zool. Soc., London **116**:611, 1946.

preserved in some areas and sharply reduced in others, while in still others they may be as completely absent as are the myelin sheaths. In some zones, indeed, there may be complete destruction of all tissue. So long as the damage primarily involves white matter, or the components of white matter, the condition may be designated as a demyelinating disease. Gray matter may be incidentally affected, as in some plaques of multiple sclerosis, but the fundamental nature of the disease is not thereby altered.

DISSEMINATED ENCEPHALOMYELITIS

The whole subject of disseminated encephalomyelitis has been rather confused. The term is used in two different senses, one indicative of inflammatory involvement of brain parenchyma, the other stressing the occurrence of demyelination. This partial dichotomy and partial overlap must be considered highly illogical. The term has, of course, a literal meaning, which is only an inflammation of brain and spinal cord that is more or less focal rather than diffuse.

In the strict sense, any fundamental distinction between "encephalitis" and "encephalomyelitis" is untenable. Both represent inflammatory processes, one involving the cerebrum alone, the other the spinal cord in addition. The difficulty has arisen in human neuropathology in the attempt to use morphologic criteria to determine the etiologic explanation. Thus, in common usage "encephalitis" implies some sort of infectious agent, while "encephalomyelitis," especially when qualified by the word "disseminated," tends to suggest a noninfectious or at least an unkown agent. In the latter category demyelination may be a prominent feature, since demyelination and inflammation may go hand in hand in some acute or subacute diseases.

Such a distinction is illogical and could readily be avoided by dealing in etiologic rather than in purely morphologic terms. The known infections of the nervous system are numerous, with quite varied manifestations. Protozoa, spirochetes, bacteria and viruses, not to mention some metazoa, can involve the brain and the spinal cord. There is no need for surprise that different viruses or different bacteria produce different histologic responses in the brain—any more than in regard to other tissues. The attack may be spotty and focal or it may be relatively diffuse. In the face of known causes the subdividing of encephalitis into "types" on morphologic grounds appears to be pure supererogation.

The difficulty arises in those inflammatory conditions the causes of which are not known. This difficulty has been compounded and aggravated by attempts to deduce the causes from morphologic changes alone. Fortunately modern neuropathology has successfully emerged from the era of interminable discussion regarding "inflammation" and

"degeneration," in which "inflammation" was somehow considered synonymous with "infection." When this was recognized as untenable, the concept of "symptomatic inflammation" was introduced. The unfortunate terms obscure the real value of the concepts.

We know that the inflammatory reaction in general is a more or less specific chemotactic response, the nature of the response being determined by the character of the chemotactic agents. These agents may be of exogenous nature, such as living infectious organisms, dead organisms or the chemical fractions of such organisms; or they may be endogenous, such as factors derived from breakdown or other injury of tissues, as in burns or trauma. Various allergic manifestations, such as the Arthus or the Shwartzman phenomenon, or the hypersensitivity leading to periarteritis nodosa, can provide the necessary chemotactic stimuli for the inflammatory response. In infectious processes the response is generally determined by both exogenous and endogenous factors. In noninfectious inflammation the endogenous factors alone are responsible.

Inflammation takes many forms, because of the enormous complexity of the constituents of tissues and of the products of their decomposition, as well as the multiplicity of exogenous agents. The subject has been widely studied in general terms, but little has been done in specific reference to the nervous system. In the brain two types of inflammation may be distinguished: One is the so-called perivascular infiltration, in which infiltrating cells seem restricted to the sheaths of blood vessels; the other, the parenchymatous involvement, with infiltration of the parenchyma. The factors responsible for these two types are not known. Concerning parenchymatous involvement, the term "inflammation" cannot, of course, be restricted merely to the presence of lymphocytes, polymorphonuclear leukocytes or plasmacytes. The mobilization of microglia cells and other histiocytic elements, producing the familiar glial nodules and other manifestations, forms a nerve tissue counterpart of the granulomatous inflammation occurring in other organs.

The relation of inflammation and demyelination is complex. In most inflammatory conditions of the nervous system due to infectious agents, demyelination is not present. Translated into familiar pathologic concepts, this means that the inflammation is not severe enough to cause destruction of tissue. Occasional uncomplicated exceptions may occur. Loss of myelin in goats, discussed on a foregoing page, is a case in point. In equine encephalomyelitis, in which we have had extensive experience with the experimental although not with the natural disease, the inflammatory changes rarely destroy myelin. Yet we have Weigert preparations of guinea pig brains in which exquisite foci of loss of myelin are occasionally evident. The time factor and the

conditions of the experiments are such as to allow only one interpretation: The direct action of the infective agent is sufficiently intense to destroy the white matter at the particular sites of virus localization. A similar explanation would apply to the "demyelination" described by Farber and associates [51] in human cases. In other words, the direct localization of the virus or the bacteria and the inflammatory response caused thereby destroyed the myelin.

Far different may be the explanation in disseminated encephalomyelitis of the dog. In some instances with demyelination the immediate presence of a virus in the brain has been proved, and it is quite possible that the mechanism is exactly comparable to that of equine encephalomyelitis. But there is no warrant for assuming that demyelination occurring in the dog is so caused in all instances. A different mechanism may be at work. Possibly in some foci in some animals the destruction may be primary and the inflammatory response relatively secondary. In other words, the particular focus of demyelination (i. e., of destruction of tissue) may be on a purely endogenous basis, although other inflammatory lesions in the same brain may possibly be due to direct localization of virus. Hurst [36] combated this concept, but his objections do not appear to us conclusive.

Thus, except under rigorously controlled conditions, the presence of a virus or other infectious agent does not prove that all demyelination present was directly caused by that virus. In the guinea pigs mentioned in a foregoing paragraph, as well as in the Listerella infection of goats discussed previously, it appears quite certain that direct action was responsible. But in most cases of the canine disease it is possible that the loss of myelin means destruction of tissue due to "allergic" or other endogenous factors. Any virus present may be an inciting or "sensitizing" factor, as well as a directly acting agent.

It is our personal belief that disseminated encephalomyelitis of the dog does not represent a single disease entity, unlike the demyelinations in monkey, sheep, horse and possibly moose. Instead, dogs exhibit widespread inflammatory lesions, some of which are caused by virus, some of which show demyelination and some of which are not caused by direct action of virus. The interrelations of these three variables are obscure. The term "disseminated encephalomyelitis" applies only to the inflammatory component, however produced, and is in no sense specific.

In regard to a classification of demyelinating diseases of animals, the disease described in dogs and goats is not in the same category as the one previously discussed. So far as demyelination in the dog or the goat

51. Farber, S.; Hill, A.; Connerly, M. C., and Dingle, J. H.: J. A. M. A. **114**: 1725, 1940.

is due to the direct local action of the virus or the bacterium, the disease is strictly analogous to a brain abscess, a tuberculoma or an infarct. That is, the loss of myelin is due to fortuitous localization of the pathogenic agent, with attendant destruction of tissue. But so far as the destruction of white matter is of purely endogenous origin (for the sake of argument, let us say it is possibly due to an "allergic" or a "sensitivity" reaction) with intrinsic predilection for white matter, the disease can be included among the true spontaneous demyelinating diseases.

CRITIQUE OF "DEMYELINATING DISEASES"

The term "demyelinating disease" has been defined, and the designated condition separated from the other conditions in which damage of white matter is also a feature. It may well be questioned whether any such distinction is truly valid, or whether it is essentially artificial.

In the study of the animal diseases discussed in the foregoing pages the strict specificity of the various conditions is impressive. Whereas in human so-called "demyelinating diseases" the nosologic outlines are vague, and by judicious selection of "transitional cases" a pseudounity may be thrown over the whole field, in lower animals such unification is not reasonable. The leukoencephalitis of horses has no counterpart in other species. In fact, the known causative agent does not act on cattle or other animals. Copper deficiency is not known to affect myelin in any species but sheep. Demyelination in the monkey bears no relation to the demyelinating disease of the dog.

An analogy may be drawn with general medicine by comparing demyelinating conditions with fevers. Fevers form an integral part of many disease pictures. In many conditions, such as pneumonia or tuberculosis, there are other equally or more important physical signs by which the disease entity is characterized. The fever is present but is merely an aspect of the total picture. To this type of fever we would compare the destruction of white matter following trauma or abscess.

On the other hand, there are many fevers unaccompanied with other physical signs, which cannot be correlated with other factors and for which no adequate explanation can be given. These, in despair, are known simply as "pyrexia of unknown origin." In such cases comparison may be drawn with "demyelinating disease" of unknown cause, such as that of the monkey.

A still different category would be fever following, say, heat stroke, in which the fever is the principal manifestation of the disease, and the "cause" is known. Comparison may be made with a primary or essential demyelinating disease the "cause" of which is known, even though the mechanisms are not. In the present state of knowledge the moldy corn disease or sway-back would fit this category.

We believe this analogy to be valid and to be in line with the opinions of Innes [15e] and of Hurst, Cooke and Melvin [86] that demyelination is not a specific process due to a single cause but represents a type of response of white matter exposed to noxious stimuli.

In terms of the analogy it would be wise to study fever either as a mechanism or as a specific disease, but not febrile diseases as a whole. In animals the specificity of the individual conditions is such that any one disease may easily be studied by itself. Animal diseases also allow for ready experimentation.

The other aspect, demyelination as a mechanism, has not received sufficient attention. We assume that destruction of myelin or, preferably, damage of white matter, represents a degrading of lipids, proteins, lipoproteins and other constituents by enzymatic action, with definite specificity of both enzymes and substrates. It is not to be wondered that an enzyme system of the horse should differ from that of the sheep. It is not enough, in lower animals, to study "demyelination" in general. The process is different in each animal, and each species presents problems of its own.

Within a given species, however, there is no indication that various forms of myelin loss, or damage of white matter, are mediated by the same enzymatic processes. Only horses are subject to "moldy corn disease." But horse brain shows destruction of white matter in the course of abscess, trauma or secondary degeneration. The assumption that the disintegration of myelin is chemically the same in these various conditions is purely gratuitous. A priori considerations would lead one to expect very different histochemical reactions if the damage were caused by moldy corn or by infection or by trauma.

Unfortunately, in the past the methods of studying chemical reactions of the brain have been limited, and histochemistry has been a backward subject in neuropathology. There have been various staining reactions and histologic sequences which have engaged attention, and which have been used with some success, especially in the study of myelin loss in secondary degeneration. But the methods are relatively crude. Fortunately, in modern anatomy and general pathology, newer and more exact histochemical technics are being elaborated, with stress on unfixed material prepared by freezing-drying technics, ultraviolet absorption curves, employment of purified enzymes, solubility studies and the like. Much valuable work has already been done on the nervous system, and it is only a matter of time before these technics are elaborated and gain wider acceptance.

It is hoped that the use of the newer technics and the analysis of variations of the process of demyelination will throw more light on those individual diseases in which damage to white matter is either a minor or a major component.

SUMMARY

In this critique a demyelinating disease is defined as one in which the disease process attacks primarily and essentially the white matter of the brain. Using such a criterion one finds several important diseases of lower animals which appear to be discrete nosologic entities. In monkeys and apes a condition has been observed, of subacute to chronic course, characterized by demyelination affecting brain, optic nerve and tracts, and spinal cord. No infectious or transmissible agent has ever been demonstrated. Dietary or other environmental factors are suggested as causal, but proof is lacking.

Through the work of Innes and of Bennetts, a disease of fetal and young lambs has been well defined. Demyelination and, in severe cases, softening leading to cavitation form a distinct disease entity. There is overwhelming evidence that copper deficiency of the pregnant ewe plays a role in the etiology and that the disease may be prevented by supplementary copper feedings. But the mechanisms at work are not known.

In horses a destructive process affecting the white matter of the cerebral hemispheres, of fulminating character, has been studied. Etiologically it has been definitely traced to the ingestion of moldy corn and appears to be of "toxic" nature.

In moose of both Maine and Minnesota demyelination of spotty character has been observed, with well defined glial scars appearing in the white matter of the cerebral hemispheres. The clinical syndrome is not clearcut, no transmissible agent has been found and the cause is obscure.

Encephalomyelitis of dogs and of goats has been described, with variable destruction of white matter. This damage is in part caused by localization of infectious agents (Listerella in goats, distemper virus or some virus like it in dogs). But in some dogs no infectious agents can be traced, and it appears unquestionable that in the dog more than one mechanism is at work to cause the damage of the white matter.

The importance of destruction of white matter as a process, rather than as a disease, is stressed.

Notes and News

Meetings.—The first annual meeting of the International Cancer Research Commission, formed in St. Louis in 1947, has been tentatively scheduled for July 15 to 22, 1949, in Paris, France. Further information may be obtained from Ignacio Millan, chairman, Avenida Veracruz 69, México, D. F. The Commission, which originally consisted of one representative from each of forty nations, has since been joined by representatives of Finland, Iceland, Israel and New Zealand.

Transactions of the Fourth International Cancer Research Congress (Sept. 2 to 7, 1947) are being published in five parts, according to an announcement by E. V. Cowdry, president of the Congress. The first part, consisting of 267 pages, including illustrations, tables and brief summaries of all papers in English, French, German, Italian, Russian and Spanish, appeared in 1948 as no. 1, vol. 6 of ACTA (Union Internationale Contre le Cancer). The other parts of the transactions will soon be available. Cost of the complete set will be $25 ($5 for each part), and postal money orders for subscriptions should be sent to J. H. Maisin, editor, 61 Voer des Capucins, Louvain, Belgium. Reprints of the separate papers can also be obtained from him.

The Second Inter-American Congress on Brucellosis will be held in Washington, D. C. in October 1950.

Deaths.—William Augustus Evans, pathologist and formerly health commissioner of Chicago, died at Muldon, Miss., Nov. 8, 1948, aged 83, of heart disease. He received his medical degree from Tulane University of Louisiana, New Orleans, in 1885, and moved to Chicago, where from 1891 on he was demonstrator of pathology, and from 1895 to 1908 professor, at the College of Physicians and Surgeons of Chicago, now the school of medicine of the University of Illinois. In 1908 he joined the faculty of Northwestern University Medical School, where at the time of his death he held the title of professor of public health emeritus. In 1907 he was appointed health commissioner of Chicago, serving until 1911, when he became health editor of the *Chicago Daily Tribune*, where for twenty-three years he conducted the column on "How to Keep Well." Among his honorary degrees was D.P.H. (doctor of public health) from the University of Michigan, Ann Arbor, in 1911. Dr. Evans presented his home town of Aberdeen, Miss., with a library of some 15,000 volumes and 10,000 manuscripts as a memorial to the Evans family, which for generations lived in that section of the state. He also restored the old home of Jefferson Davis, "Beauvoir," in Biloxi, Miss., converting it into a public building now maintained by a Davis memorial association.

Gustave Ricker, for many years director of the Pathologic Institute in Magdeburg, Germany, and one of the outstanding and original European pathologists of the first half of this century, died at the age of 77 in Dresden, on Sept. 23, 1948. His famous book on the pathology of relations[1] was published in 1924. Ricker

1. Ricker, G.: Pathologie als Naturwissenschaft, Relationspathologie; für Pathologen, Physiologen, Mediziner und Biologen, Berlin, J. Springer, 1924.

postulated that all stimuli act primarily on the nervous system, and that all physiologic and pathologic responses in tissues and organs are dependent on changes of the blood and lymph circulation which are under nervous control. His experiments in which the circulation of the pancreatic region of the rabbit was studied under various degrees of irritation resulted in the establishment of his widely known *Stufengesetz* (law of degrees) and furnished a foundation for the understanding of many functional disturbances of the circulation and their sequelae. While Ricker was an outsider for a long period, frowned on by numerous advocates of cellular pathology, much of his theory has been more and more accepted during the last two decades. His doctrines will stimulate further discussion and research for many years to come.—KARL T. NEUBUERGER.

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